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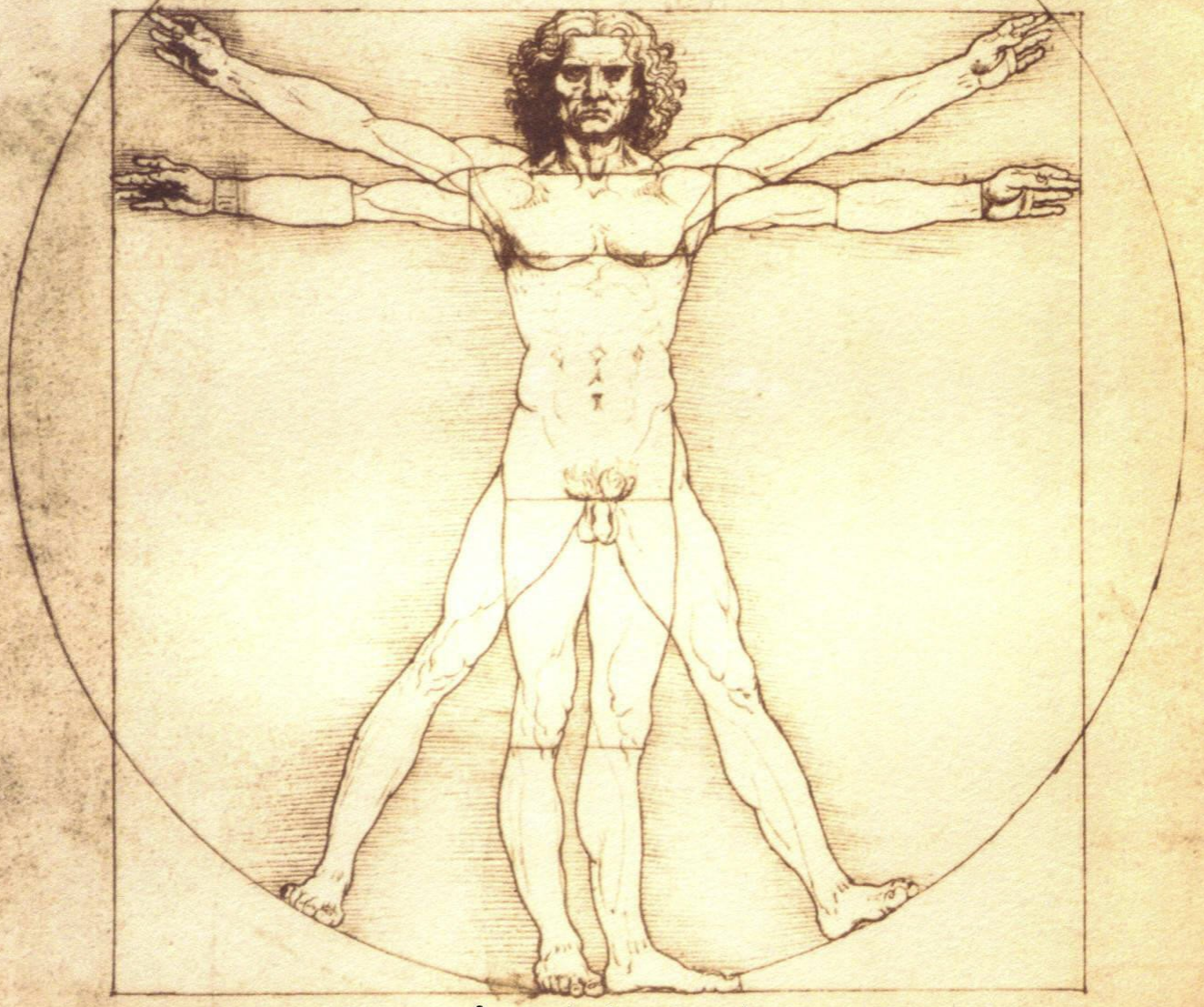

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Hygeia was the Greek goddess of health and she was worshiped in connection with Aesculapius, her father, the god of medicine and health. She is said to be again, the grand daughter of the God, Apollo. She is also the sister of Panakeia or Panacea (means all-cures) Akeso (Goddess of Healing), and Iaso (which means Remedies). Hygeia is usually depicted as a young woman, who holds a sacred snake (symbolizes resurrection), which is often combined with the rod of Asclepius to form the cadacus, or symbol of medicine. Often this snake is portrayed as drinking from a cup (symbolizing medicine), which has become known as the pharmacist's bowl.

Originally, she was the guardian of physical health and later became the goddess of mental health, as well. Eventually, she became a protectress against various kinds of danger, an attribute which she shared with Aesculapius. It is from Hygeia, the word hygiene originates. Hygiene is the science of preserving health. The subject of hygiene includes all of the agencies affecting the physical and mental well being of people. In its public aspects, it is concerned with soil; climate; character; materials and arrangement of dwellings; heating and ventilation; removal of wastes; medical knowledge on the incidence and prevention of disease; and the disposal of the dead.

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FORMULATION AND EVALUATION OF VENLAFAXINE HCl MICROSPHERES

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IN-VITRO ANTIMICROBIAL EFFECTS OF SOME SELECTED PLANTS AGAINST BOVINE MASTITIS PATHOGENS

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Ethno medicinal Plants for Cancer therapy – A review

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Abstract

A huge reservoir of bioactive compounds exists in many species of plants of Earth, only a small percentage of which have been examined and continued to be an important source of anticancer agents. Worldwide effects are ongoing to identify new anticancer compounds from plants. With the current decline in the number of new molecular entities from the pharmaceutical industry, novel anticancer agents are being sought from traditional medicines. This article reveals a detailed review of ethno medicinally important plants in cancer from Indian medicinal plants which will be useful to treat various types of cancer. It will be helpful to explore the medicinal value of the plants and for the new drug discovery from them for the researchers and scientists around the globe.

Key words: Cancer, Medicinal plants, bioactive compounds, Anticancer.

1. Introduction

Cell growth and cell multiply process is known as cell division. It must be extremely controlled that all the cells in the body should grow at the right place, and for all the organs and tissues to function properly. When the cells divide too quickly, consequences can be disastrous. When a cell divides, it first makes an exact copy of its DNA via a process called DNA replication, before splitting into half, to form two 'daughter' cells, that are genetically identical. Hundreds of proteins involve in Cell division. Some proteins inform the cell when or when not to divide. Others were responsible for making sure that the DNA is copied accurately. Yet more were involved physically by pulling the duplicated chromosomes apart as the cell to split into two. Uncontrolled cell division may have many causes, to form any type of cell. But usually results from defects or damage from one or more of the genes involved in cell division.

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When those genes were damaged (mutated) on some way, for instance on exposure to cigarette smoke or ultraviolet radiation, the cell may start dividing uncontrollably. Those defective cells might multiply to form a lump of abnormal tissue called a tumour.

1.1. **Cancer-causing genes**

There are four main types of gene involved in cell division. Most tumours have faulty copies of more than one of the genes viz., Oncogenes - Ontogenesis were the genes, under normal circumstances, that play a role tell the cells to start dividing. When oncogenes are activated, they speed up a cell's growth rate. When one of them becomes damaged, causing cancer, it is like the accelerator is becoming stuck down - the cell, and all its daughter cells, are permanently instructed to divide. *Tumour suppressor genes*- This gene was co-discovered in 1979 by the scientist Professor Sir David Lane Cancer Research UK. These genes make proteins whose normal function is the opposite to that of ontogenesis. One of the most important tumour suppressor genes is called p53. *Suicide genes* - Apoptosis or cell suicide, which was a highly complex and hugely important process. Cells usually have the ability to commit suicide whenever something goes wrong, to prevent damage to their neighbours. There are many different genes involved. If the 'suicide genes' become damage, then a faulty cell can keep dividing and become cancerous. *DNA-repair genes* - The DNA in every cell in the body is under constant assault from a variety of directions. But cells contain many different proteins whose job is to repair the damaged DNA. Thanks to those, scientists think that the vast majority of DNA damage is repaired immediately, with no ill effects. But if the DNA damage occurs to a gene that makes a DNA repair protein, a cell's ability to repair itself will be reduced, and that can allow errors to accumulate in other genes over time. Causes of cancer as followes.

1) Viruses such as *Epstein-Barr-Virus* (EBV), *Hepatitis-B-Virus* (HBV), *Human Papilloma Virus* (HPV).
2) Environmental and occupational exposure such as ionizing, UV radiation, exposure to chemicals including vinyl chloride, benzene and asbestos.
3) Life style factors such as high-fat, low fiber diets, tobacco, ethanol etc.
4) Medication such as alkylating agents and immunosuppressant's.
5) Genetic factors such as inherited mutations, cancer causing genes, defective tumor suppressor genes.

1.2. **Free radicals and cancer**

A free radical is nothing more than a molecular structure which contains an unpaired electron. Electrons tend to stay in pairs. Electron pairs make up the chemical bonds which keep molecules from flying apart. An unpaired electron,driven by a potent chemical force which compels it to find a mate.

Thus molecular instinct that merges with another electron was so powerful that the searching molecule behaves erratically, moving about much like a weapon within cellular structures. Its random and wild molecular movements within cellular material can create cellular damage, which can eventually result in degeneration or mutation.

A free radical can destroy a protein, an enzyme or even a complete cell. To make matters worse, free radicals can multiply through a chain reaction mechanism resulting in the release of thousands of the cellular oxidants. When it happens, cells can become so badly damaged that DNA codes can be altered and immunity can be compromised. Contact with a free radical or oxidant on the scale can create cellular deterioration, resulting in diseases like cancer. Tissue breakdown from the oxidative stress can also occur, which contributes to aging, arthritis and a whole host of other degenerative conditions. Our constant bombardment with free radicals had been likened to being irradiated at low levels all the time. Unfortunately, because of the damage free radicals cause within our cellular structures, the sad fact was that many of us will die prematurely from one of a wide variety of degenerative diseases. Free radical damage has been associated with over 60 known diseases and disorders. An important fact to remember that the act of breathing oxygen activates those reactive chemical structures known as free radicals. To make matters worse, as because in our generation more than any other had been exposed to a number of potentially harmful environmental substances, free radical formation can reach what has been referred to as epidemic proportions. Some of the more dangerous free radical producing substances include: cigarette smoke, herbicides , high fats, pesticides, smog car exhaust , certain prescription drugs, diagnostic and therapeutic x-rays , ultra-violet light , gamma radiation , rancid foods , certain fats , alcohol some of our food and water supplies , stress, poor diets etc. Even exercising, as beneficial as it is, can initiate the release of free radicals within our cellular systems. Aerobic exercising produces damaging oxidation by-products. Many of these are not completely neutralized by internal safety mechanisms and an overload can occur. Supplementing the diet with effective antioxidant compounds.

Numerous research studies support the fact that many cancers, in particular breast cancer- diet related. Moreover, the risks of certain kinds of cancer could be significantly reduced with dietary changes. While most of us are aware of the wonders of a low-fat diet, a tremendous amount of data conceding other cancer preventative nutrients never reaches the average consumer. For instance, recent studies suggested that just reducing dietary fat may not be enough to prevent certain cancers.

Perhaps more and more research suggested that, lack of certain protective nutrients appeared to originate from dietary sources that increase risk of cancer and other degenerative diseases. The role of certain bioflavonoid compounds were the exceptional free radical scavengers that just begin to emerge, and the protective potential of those flavonoids was impressive, to say the least.

1.3. **Cancer - Indian scenario**

Every year about **8,50,000** new cancer cases being diagnosed, India resulting about **5,80,000** cancer related death every year. India had the highest number of the oral and throat cancer cases in the world. Every third oral cancer patient in the world is from India. In males Oral, Lungs and Stomach cancers was the three most common causes of cancer incidence and death whereas In females Cervical, Breast and Oral cancers were the three main causes of cancer related illnesses and death. Overall cervical cancer was the number one cause of cancer death in India. That was really unfortunate as cervical cancer can be easily prevented and also relatively easy to diagnose and treat at an early stage. Compared to developed countries overall there were less cancer cases in India but that could be due to under diagnosis and under reporting. At the same time regional, ethnic, dietary and socio-economic factors might also results in difference in the cancer susceptibilities and the incidence. Also cancer was mainly a disease of old ages. World wide median age at diagnosis was about 60 years. Average life span was about 58 yrs in India compared to 75 yrs in the developed world.

1.4. **Cancer- global scenario**

Among all the cancer, Lung cancer is the most common worldwide and accounts for major death annually. The following Table 1 shows the global scenario for various types of cancer.

The three leading cancer killers were different than the three most common forms, (i) Lung cancer responsible for 17.8 per cent of all cancer deaths. (ii) Stomach 10.4 per cent and (iii) Liver 8.8 percent. Industrial nations with the highest overall cancer rates include: U.S.A, Italy, Australia, Germany, The Netherlands, Canada and France. Developing countries with the lowest cancer were in Northern Africa. Cancer rates could further increase by 50% to 15 million new cases in the year 2020.According to the World Cancer Report, the most comprehensive global examination of the disease to date. However, the report also provides clear evidence that healthy lifestyles, and public health action by governments and health practitioners could stem this trend, thus prevent as many as one third of cancers worldwide.

1.5. **Plant phytochemicals on cancer – an overview**

Plant materials was been used for the treatment of malignant diseases for centuries. Recent phytochemical examination of plants which have a suitable history of use in folklore for the treatment of cancer had induced often resulted in the isolation of principles with antitumour activity. An intensive survey of plants, micro organism and marine animals for antitumour activity began in the later 1950s mainly because the United States National Cancer Institute (NCI) instigated and fund a major screening programme. Random selection screening programme was adopted, since novel compounds may be found anywhere from plant or animal kingdom.

Soybean phytochemicals such as genistein (4',5,7-tribydroxy isoflavone) inhibit the growth of transplantable human prostate carcinoma.[1] Epidemiological studies have consistently shown that regular consumption of fruits and vegetables strongly associated with reduced risk of developing chronic diseases such as cancer as the phytochemical extracts from it exhibit strong antioxidant activity.[2] Andrographolide the potential cancer therapeutic agent isolated from *Andrographis paniculata.*[3]

In the screening of Yemeni plants used in folk medicine for the anticancer potential, the methanolic extracts of *Dendrosicyos Socotrana, Withania aduensis, Withania riebeckii, Dracena Cinnabari* and *Buxus hildebrandlii* exhibited the highest toxicity on all tumor cell lines.[4] The four varieties of muscadine grape extract had the ability to inhibit the activitiy of matrix metalloproteinases implying that those could be good inhibitors of carcinogenesis.[5] The limonoids isolated from the methanol extract of *Khaya Senegalensis* proved good anticancer activity.[6] The leaf extract of Ashwagandha selectively killed tumor cells and thus it was a natural source for safe anticancer medicine.[7] The fruit of deerberry (*Vaccinium stamineum*) exhibited the anticancer capability of human lung and leukemia cancer cells.[8] Polyphenolic extracts from *Vaccinium macrocarpon* inhibited the growth and proliferation of breast, colon, prostate, lung,
and other tumors as do flavonols, proanthocyanidin, oligomers, and triterpenoids isolated from the fruits of the same.[9]

Morinda citrifolia showed of cancer preventive effective on both clinical practice and laboratory animal models.[10] An alcoholic extract of *Biorhythms sensitivum* for antitumor activity could inhibit the solid tumor development on mice induced with Dalton's lymphoma ascites (DLA) cells and increase the life span of mice bearing Ehrlich ascites carcinoma (EAC) tumors.[11] Edible fruits and berries served the source for novel anticancer agents, given that extracts of those foods have demonstrated cytotoxic activity against tumor cell lines.[12] Nimbolide, a triterpenoid extract from the flowers of the neem tree was found to have antiproliferative activity against some cancer cell lines[13]. *Semecarpus anacardium* Linn nut milk extract exerts its anticancer effect through quenching - reactive oxygen species.[14] The cytotoxic activities of two medicinal herbs *Linum persicum* and *Euphorbia cheradania* that are native to Iran showed cytotoxic activity on tumor cell lines.[15] The Pomegranate extracts inhibits the growth of breast cancer cells.[16] Brassinosteroids, steroid plant hormones are promising leads for potential anticancer drugs.[17] The *careya arborea* bark significantly reduced the solid tumor volume induced by DLA cells.[18] The methanol extract of *Bauhinia racemosa* stem bark exhibited antitumor effect in EAC bearing mice.[19] The antitumor activity of the ethanol extract of *Indigofera aspalathoides* was established.[20]

The extract of 12 Chinese medicinal herbs such as *Anemarrbena asphodeloides* (Root), *Artemisia argyi* (leaf), C*ommiphora Myrrh* (Resin), *Duchesnea indica* (Aerial Plants), *Gleditsia sinessis* (Fruit), *Ligustrum lucidum* (fruit), *Rheumpalmatum* (Root and Rhizome),

Rubia cordifolia (Root), Salvia Chinesis (Aerial parts), *Scutellaria barbata* (Aerial Parts), *Uncaria rhychopylla* (Stem), *Vaccaria segetails* (seed) showed anticancer effects invitro and those effects were markedly greater on cancer cells compared with normal cells.[21]

Phytoconstituents extracted from a large number of plants belonging to the genus Hypericum are known to possess potent anticancer nature[22] cytotoxic activity of *Sarris cernuss* extract on human colon and breast carcinoma cultures was proved.[23] The natural antioxidant gallic acid (GA) isolated from the fruits of an Indonesian medicinal Plant, *Phaleria Macrocarpa* was proved to be a potent anticancer compound.[24] The rhizome *Zingiber Officinalis*, one of the most widely used species of the ginger family is a common condiment for various foods and beverages. The pungent vallinoids i.e., 6-gingerol and 6-paradol, shogaolsand zingerone attributed to the anticancer properties of ginger .[25]

The antineoplastic activity of methanolic extracts of five medicinal plants that are native to Iran including *Galium mite*, *Ferula Angulat*a, *Stachys obtuscrena*, *Grsium bracteosum*, and *Echinophora Cinerea* was investigated and proved to have anti tumor activity.[26] *Panax ginseng* and its extracts have long been used for medical purposes and there increasing interest in developing ginseng products as cancer preventive agents.[27] Purified bioactive compounds derived from medicinal mushrooms were potentially important for new source of anticancer agents.[28]

The Saponins from the plant of china, *clematis manshrica* has obvious antitumor effects against various transplanted tumor on mice.[29] The Embelin derivatives such as 1,4 - benzoquinone derivative 5-0 ethyl embelin(1) and 5-0 methyl embelin are promising antimitotic and anti cancer molecules.[30] Sesquiterpenes the class of naturally occuring molecules that are 15-carbon isoprenoid compounds. Those typically found on plants and marine life. They have therapeutic potential in decreasing the progression of cancer.[31]

The anticancer activity from *Platycodon grandiflorum* was proved and established.[32] The methanol extract of stem bark of *Dillenia pentagons* appears to be more active against Dalton's lymphoma.[33] *Limonium Vulgare*, Artemisia *Maritima* and *Salicornia europaea* showed antineoplastic activities. The extracts of *Ononis spinosa*, *Trifolium fragiferum* and *Trifolium repen* showed tumor growth inhibiting activities.[34] Methanol extract *Ledum groelandicum* Retzius (Labrador tea) leaf twig extract showed anticancer activity.[35] The anti-neoplastic activity of guduchi (*Tinospora cordifolia*) on Ehrlich ascities carcinoma was proved.[36] Some of the other plants of anticancer activities are shown in Table-2.

Table -1: Global Scenario on Types of Cancer.

S.No	Type of Cancer	No. of Patients affected /year
1.	Lung	1.2 million
2.	Breast	Over 1 million
3.	Colorectal	9,40,000
4.	Stomach	8,70,000
5.	Liver	5,60,000
6.	Cervical	4,70,000
7.	Esophageal	4,10,000
8.	Head and Neck	3,90,000
9.	Urinary Bladder	3,30,000
10.	Malignant Non-Hodgkin lymphomas	2,90,000
11.	Leukemia	2,50,000
12.	Prostate and Testicular	2,50,000
13.	Pancreatic	2,16,000
14.	Ovarian	1,90,000
15.	Kidney	1,90,000
16.	Endomaterial	1,88,000
17.	Nervous system	1,75,000
18.	Melanoma	1,33,000
19.	Thyroid	1,23,000
20.	Pharynx	65,000
21.	Hodgkin disease	62,000

Table-2: Indian Medicinal plants having anticancer activity

S.no.	Name of the plant	Family	Parts used
1.	*Calotrophis gigantea*	Asclepiadaceae	Whole plant
2.	*Cajanus cajan*	Fabaceae	Leaves
3.	*Butea monosperma*	Fabaceae	Bark
4.	*Bauhinia variegata*	Caesalpinaceae	Root
5.	*Bacopa monnieri*	Scropulariaceae	Whole plant
6.	*Azadirachta indica*	Meliaceae	Bark
7.	*Asparagus racemosus*	Liliaceae	Root
8.	*Aphanamixis polystachya*	Meliaceae	Bark
9.	*Aloe barbadensis*	Liliaceae	Leaf juice
10.	*Alium cepa*	Liliaceae	Bulb
11.	*Acorus calamus*	Araceae	Rhizome
12.	*Cassia absus*	Caesalpinaceae	Leaves
13.	*Cassia auriculata*	Caesalpinaceae	Root
14.	*Cassia senna*	Caesalpinaceae	Leaves
15.	*Catunaregum spinosa*	Rubiaceae	Bark/Fruit
16.	*Citrullus colocynthis*	Cucurbitaceae	Root
17.	*Citrus medica*	Rutaceae	Root
18.	*Cissus quadrangularis*	Vitaceae	Whole plant
19	*Clerodendrum serratum*	Verbanaceae	Root
20	*Clerodendrum viscosum*	Verbanaceae	Leaves
21	*Crinum asiaticum*	Amaryllidaceae	Bulb
22.	*Daucus carota*	Apiaceae	Root
23.	*Embelia ribes*	Myrsinaceae	Fruit
24	*Flacourtia jangomos*	Flacourtiaceae	Bark/Leaf
25	*Jatropha curcas*	Euphorbiaceae	Leaves,seed,oils
26	*Kaempferia galanga*	Zingiberaceae	Rhizome
27	*Kaempferia rotunda*	Zingiberaceae	Tubers
28	*Lanata camara*	Verbanaceae	Whole plant
29	*Lens culinaris medikus*	Fabaceae	Seed
30	*Limonia acidissima*	Rutaceae	Fruit
31	*Macrotyloma uniflorum*	Fabaceae	Seed
32	*Mimosa pudica*	Mimosaceae	Whole plant
33	*Nicotiana tabacum*	Solanaceae	Leaves
34	*Operculina turpethum*	Convolvulaceae	Root
35	*Rhinacanthus nasuta*	Acanthaceae	Whole plant
36	*Salvadora persica*	Salvadoraceae	Bark,Leaf,Shoot,Fruit
39	*Symplocus cochinchinensis*	Symplocaceae	Bark
40	*Tylopora indica*	Asclepiadaceae	Root, Leaf
41	*Vernonia cinerea*	Asteraceae	Whole plant
42	*Vitex trifolia*	Verbanaceae	Leaf
43	*Zanthoxylum armatum*	Rutaceae	Bark,Fruit
44	*Xanthium strumarium*	Compositae	Root

2. **Conclusion**

From the present review, it can be concluded that cancer is the leading cause of death in developing countries like India. As there is an enormous increase in the population day by day, the alternative therapy in the market is getting its glimpse. The cheap herbal drug treatment may highly be recommended to the rural and poor people to treat effectively the cancers of various type is an ideal choice. Based on that the siddha medicines are coming up in combination with metals and other essential supplements to improve the immune status of the cancer patients in India. The above survey reveals the role of Indian medicinal plants and the various phytochemicals may be treated effectively for cancer. The available literature finds to be very impressive which may give an indication for the therapeutic usefulness. Only few of the plants listed here and there are hundreds of plants unexplored need much detailed survey. The isolation, identification of active principles and pharmacological studies of the active phytoconstituents may be considered and studied elaborately to treat effectively for various types of cancer.

Acknowledgement

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Plants as Potent Anti diabetic and wound healing agents- A review

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Abstract

Wounds generally termed as physical injuries that result in an opening or breaking of the skin. There are different types of wounds which range from mild to potentially fatal. Wound healing is impaired in diabetic patients with infection or hyperglycaemia. Diabetes mellitus is one of the major contributors to chronic wound healing problems. The diabetic patients with ulcer become at high risk for major complications which include infection and amputation. In traditional medicine plants are generally used for treatment of various acute and chronic diseases and abnormalities in the body. Due to the present fast life of the humans a drastic increase in chronic disease conditions mainly diabetes has been determined. Most of these patients tend to face a tremendous problem when they get an infected wound. Hence in the current review a list of the plants used in traditional medicine for the treatment of wounds and diabetes were screened. The work includes a list of traditionally claimed plants used for diabetes and wounds which are scientifically proved as well as scientifically not proved.

Key words: Inflammatory, Proliferative, Remodelling, Diabetes mellitus, Homeostasis

1. Introduction

Plants have anchored to the mother earth long before man has set his feet and it is said that god had endowed them with materials for survival of man and animal long before these creatures were made by him[1]. The world health organization (WHO) estimates that about 80% of the population is still depends upon these herbal medicines for their treatment of diseases due to easy availability, economic and less side effects when compared to allopathic system of medicines. Nearly 2000 of natural drugs are mentioned in Indian Materia Medica that have reported various pharmacological activities, out of these 1600 are from plant origin.[2] Herbal remedies have formed the basis of traditional medicine for millennia, and have formed the root of modern pharmacology. While science from roughly the 1880's onwards has striven to isolate the active compounds found in medicinal herbs, the list is ever growing[3]. Wound infections are most common in developing countries, such as Sub-Saharan African and South Asian countries, than in developed countries. Current estimates indicate that nearly 6 million people suffer from chronic wounds worldwide [4].

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The prevalence of chronic wounds in the community was reported as 4.5 per 1000 population, whereas that of acute wounds was nearly double, at 10.5 per 1,000 populations [5]. Plant products are potential agents for wound healing, and largely preferred because of their widespread availability and effectiveness as crude preparations. Due to the present stress filled life a lot of people are developing diabetes at a very younger age. It was reported that a lot of children too are developing this chronic and fatal disorder. Hence this encouraged to develop a list a plants which posses the power to heal these conditions.

1.1. **Over view of wound**

A wound is one in which the skin or another external surface is torn, pierced, cut, or otherwise broken[6]. It can be classified into two type ; open wound and a closed wound[7] .There are many different types of wounds ranging from mild to severe to potentially fatal. Contusions, small incisions, and abrasions tend to be non-threatening, though some may pose the risk of infection. Deep punctures, avulsions, and amputations, however, may be life threatening. In most cases, the risks posed by all types of wounds differ in severity based on the instrument causing them, the ease of blood flow, and the cleanness or jaggedness of the edges of the damaged skin.Abrasions, Avulsions, Contusions, Crush wounds, Cuts, Incised wound, Lacerations, Penetrating wound, Punctures are few examples of types of wound [8,9]

Phases of wound

Bleeding phase[10]

Bleeding phase is relatively short lived depend in the nature of the wound and the intensity of the wound and the vascular system available at the site if the wound.

Inflammatory phase[10]

The inflammatory phase prepares the area for healing and immobilizes the wound by causing it to swell and become painful, so that movement becomes restricted. The fibroblastic phase rebuilds the structure, and then the remodelling phase provides the final form.

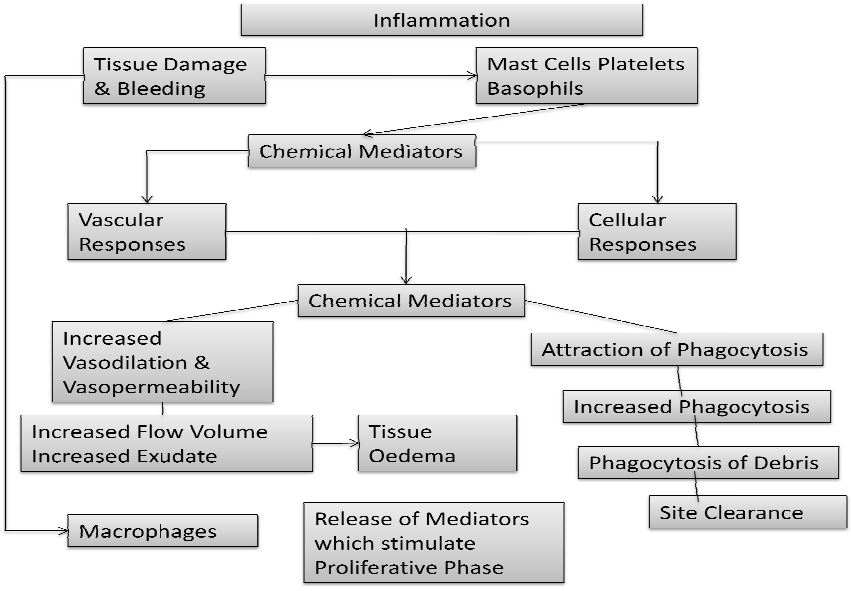

Figure 1**:** Events in the Inflammatory Phase

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A REVIEW ON MALE FERTILITY

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Abstract

Spermatogenesis is a complex process that involves stem-cell renewal, genome reorganization and genome repackaging, and that culminates in the production of motile gametes. Problems at all stages of spermatogenesis contribute to human infertility. Male infertility, is characterized by hypogonadism, decreased semen quality or ejaculatory dysfunction, accounts for approximately 20% of infertility cases. The main cause for infertility apoptosis. Apoptosis, a form of programmed cell death in contrast to necrosis, which represents "accidental cell death," plays an important role in spermatogenesis. excessive alcohol intake resulted in Leydig cell apoptosis, while cocaine resulted in mitochondrial apoptosis, excessive alcohol intake resulted in Leydig cell apoptosis, while cocaine resulted in mitochondrial apoptosis. Men with severe oligozoospermia less than 1 to 5 x 10^6 sperm/ml are considered as a male infertility by diagnosis. Studies on male infertility, potentially opening the door for treatment advances for improving spermatogenesis. Newer techniques like sperm retrieval and VE modifications are promising and becoming increasingly more popular for the estimation of damage of certain DNA is responsible for male infertility.

Key words: Male Infertility, Diabetes, Sperm DNA, Impotence.

1. Introduction

Infertility primarily refers to the biological inability of a person to contribute to conception. Infertility may also refer to the state of a woman who is unable to carry a pregnancy to full term. There are many biological causes of infertility, some which may be bypassed with medical intervention. Male infertility is a reasonably common problem. Being infertile has nothing to do with male sexual prowess (virility), but rather with the absence of healthy sperm in the semen that are capable of travelling to meet the ovum . Infertility is of four types as follows.

Infertility : Reproductive endocrinologists, the doctors specializing in infertility, consider a couple to be infertile if: The couple has not conceived after 6 months of contraceptive-free intercourse if the female is over the age of 35 (declining egg quality of females over the age of 35 account for the age-based discrepancy as when to seek medical intervention).

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Alternatively, the NICE guidelines define infertility as failure to conceive after regular unprotected sexual intercourse for 2 years in the absence of known reproductive pathology.

Sub fertility:

A couple that has tried unsuccessfully to have a child for a year or more is said to be subfertile meaning less fertile than a typical couple. The couple's fecundability rate is approximately 3-5%. Many of its causes are the same as those of infertility. Such causes could be endometriosis or polycystic ovarian syndrome.

Primary vs. secondary infertility:

Couples with primary infertility have never been able to conceive,[4] while, on the other hand, secondary infertility is difficulty conceiving after already having conceived (and either carried the pregnancy to term or had a miscarriage). Technically, secondary infertility is not present if there has been a change of partners.

EPIDEMIOLOGY:

Approximately 15-20% of couples attempting to achieve pregnancy in the United States each year face difficulties with fertility. Of those couples, a pure "female factor" is responsible for about 35-40% of cases. About another 35% of cases are pure "male factor." Couples with a combination of male and female factors account for the remaining 25-30% of cases. Therefore, a male infertility factor plays a part for more than 50% of couples unable to conceive on their own. These numbers stress the need for appropriate male factor evaluation and treatment options.

2**. CAUSES OF INFERTILITY:**

More than 90% of male infertility cases are due to low sperm counts, poor sperm quality, or both. The remaining cases of male infertility can be caused by a number of factors including they are:

Environmental pollutants, Exposure to high heat for prolonged periods,Genetic abnormalities
Heavy use of alcohol, marijuana, or cocaine, smoking.,Hormone deficiency or taking too much of a hormone,Impotence,Infections of the testes or epididymis,Older age,Previous chemotherapy,Previous scarring due to infection, trauma, or surgery,Radiation exposure,Retrograde ejaculation,Smoking,Surgery or trauma,Use of prescription drugs, such as cimetidine, spironolactone, and nitrofurantoin

Sperm Abnormalities:

Sperm abnormalities can be caused by a range of factors, including congenital birth defects, disease, chemical exposure, and lifestyle habits. In many cases, the causes of sperm abnormalities are unknown.Sperm abnormalities are categorized by whether they affect sperm count, sperm movement, or sperm shape they include:

Low Sperm Count (Oligospermia)**:** A sperm count of less than 20 million/mL is considered low sperm.

Azoospermia refers to the complete absence of sperm cells in the ejaculate, and accounts for 10 - 15% of cases of male infertility. Partial obstruction anywhere in the long passages through which sperm pass can reduce sperm counts. Sperm count varies widely over time, and temporary low counts are common. Therefore, a single test that reports a low count may not be a representative result.

Poor Sperm Motility (Asthenospermia)**:** Sperm motility is the sperm's ability to move. If movement is slow, not in a straight line, or both, the sperm have difficulty invading the cervical mucous or penetrating the hard outer shell of the egg. If 60% or more of sperm have normal motility, the sperm is at least average in quality. If less than 40% of sperm are able to move in a straight line, the condition is considered abnormal. Sperm that move sluggishly may have genetic or other defects that render them incapable of fertilizing the egg. Poor sperm motility may be associated with DNA fragmentation and may increase the risk for passing on genetic diseases.

Abnormal Sperm Morphology (Teratospermia)**:** Morphology refers to shape and structure. Abnormally shaped sperm cannot fertilize an egg. About 60% of the sperm should be normal in size and shape for adequate fertility. The perfect sperm structure is an oval head and long tail.

Retrograde Ejaculation:

Retrograde ejaculation occurs when the muscles of the bladder wall do not function properly during orgasm and sperm are forced backward into the bladder instead of forward out of the urethra. Sperm quality is often impaired.

Retrograde ejaculation can be the consequence of several conditions:

Surgery to the lower part of the bladder or prostate (the most common cause of retrograde ejaculation), Diseases such as diabetes and multiple sclerosis, Spinal cord injury or surgery, Medications such as tranquilizers, certain antipsychotics, or blood pressure medications also may cause temporary retrograde ejaculation, Aging.

Genetic Disorders:

Certain inherited disorders can impair fertility. Examples include:

Cystic fibrosis can cause missing or obstructed vas deferens.

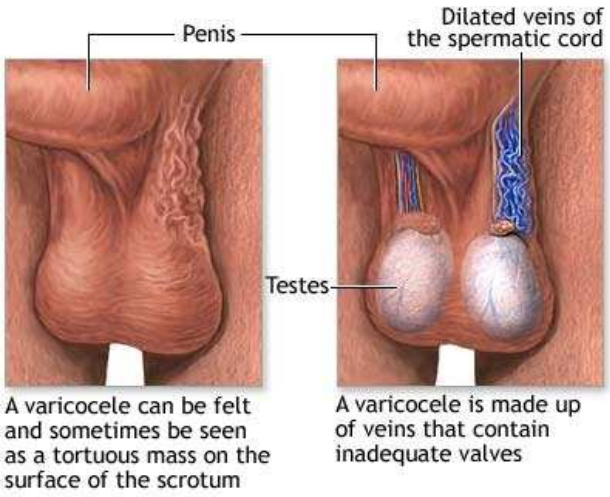
Polycystic kidney disease, a relatively common genetic disorder that causes large cysts to form on the kidneys and other organs during adulthood, may cause infertility as the first symptom if cysts develop in the reproductive tract.

Klinefelter syndrome is marked by two X and one Y chromosomes, which leads to the destruction of the lining of the seminiferous tubules in the testicles during puberty, although most other male physical attributes are unimpaired.

Kartagener syndrome, a rare disorder that is associated with a reversed position of the major organs, also causes impaired sperm motility.

3. **Risk factors:**

Varicocele:



A varicocele is an abnormally enlarged and twisted (varicose) vein in the spermatic cord that connects to the testicle. Varicoceles are found in about15% of all men and in about 40% of infertile men, although it is not clear how much they affect fertility or by what mechanisms. They can raise testicular temperature, which may have effects on sperm production, movement, and shape.

Age

Age-related sperm changes in men are not abrupt, but are a gradual process. Aging can adversely affect sperm counts and sperm motility. The genetic quality of sperm declines as a man ages.

Sexually Transmitted Diseases

Repeated *Chlamydia trachomatis* or gonorrhea infections are most often associated with male infertility. Such infections can cause scarring and block sperm passage. Human papilloma viruses, the cause of genital warts, may also impair sperm function.

Lifestyle Factors

Nearly any major physical or mental stress can temporarily reduce sperm count. Some common conditions that lower sperm count, temporarily in nearly all cases, include:

Emotional Stress: Stress may interfere with certain hormones involved with sperm production.

Testicular Overheating: Overheating, such as from high fevers, saunas, and hot tubs, may temporarily lower sperm count.

Substance Abuse: Cocaine or heavy marijuana use can temporarily reduce the number and quality of sperm. Sperm actually have receptors for certain compounds in marijuana that may impair the sperm's ability to swim and also inhibit their ability to penetrate the egg. Anabolic steroid use can shrink testicles and decrease sperm production. Heavy drinking may also impair fertility.

Smoking: Cigarette smoking may affect sperm quality.

Laptops: laptops can also cause male fertility, Men who rest computers on their laps for long periods could be risking their fertility by the increase in temperature 1^0C may alter the production of the sperm. So the usage of laptops on laps should be avoided by men

Environmental Factors:

Occupational or other long-term exposure to certain types of toxins and chemicals (such as herbicides and pesticides) may reduce sperm count by either affecting testicular function or altering hormone systems. Estrogen-like and hormone-disrupting chemicals such as bisphenol A, phthalates, and organochlorines are particular potential concerns. Chronic exposure to heavy metals such as lead, cadmium, or arsenic may affect sperm quality. At this time, there is no strong evidence supporting a serious harmful effect on fertility in men who have normal limited exposure to these chemicals.

Diabetes induced male infertility:

Sperm+ Sugar= Infertility

Now a days diabetic mellitus is the most complicatory disease in the world. India one of the leading country suffering with diabetes. More number of complications will arise with diabetic mellitus e.g.: diabetic infertility. Infertility is the most causative disease which effects the reproductive organs of both male and female persons. Diabetes in men has a direct effect on fertility at a molecular level. In diabetes sperm RNA was significantly altered, once altered their ability to repair sperm DNA is collapsed and once this is damaged it cannot be restored.

4. DIAGNOSIS OF INFERTILITY IN MALE:

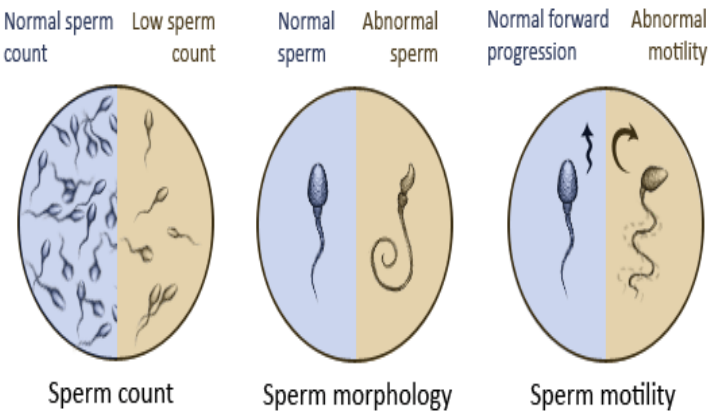
Infertility can be diagnosed by several methods:

Semen analysis:

Although semen analysis is not a test of fertility, a carefully performed semen analysis is a highly predictive indicator of the functional status of the male reproductive hormonal cycle, Most specialists collect at least three specimens in which the seminal parameters are within 20% of each other before establishing a baseline for semen quality. The semen specimen is best obtained by masturbation after a two to three day period of abstinence. Abstinence intervals give large source of variability. With each day of abstinence semen volume increases by 0.4 cc, sperm concentration by 10-15 million per cc, and total sperm count by 50-90 million.

Sperm motility and morphology appear to be unaffected by 5-7 days of abstinence, but longer periods lead to impaired motility. The minimum number of specimens to define good or poor quality of semen is three samples over a 6-8 week interval with a consistent period of abstinence of 2-3 days.

In a longitudinal analysis of semen from both fertile and infertile men, it was found that 97% of men with initial good sperm concentration would continue to show good density after as many as 3-6 specimens.

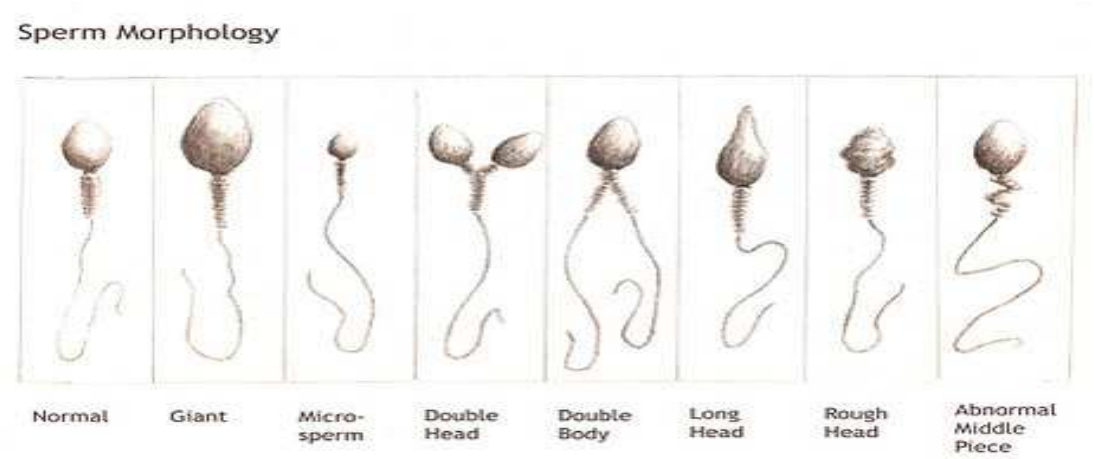


Sperm morphology:

The results of a sperm morphology exam are reported as percent normal. It is always the case that some sperm from an ejaculate are morphologically abnormal, but when that fraction becomes excessive, fertility may decrease. It is also useful to subclassify the abnormal population into the types of abnormality observed. Two types of classification schemes are commonly used: Abnormalities can be classified as affecting the head, midpiece or tail. The most basic type of classification scheme differentiates primary and secondary abnormalities:

Anatomic site of the defect*:* The problem can involve the **head, midpiece or tail**. Some abnormal sperm may have defects in more than one site.

Primary versus secondary defects*:* Primary defects are the more severe and are thought to originate while the sperm was still within the semeniferous epithelium of the testis. Secondary defects are less serious and thought to arise during passage thought the epididymis or by mishandling after ejaculation.



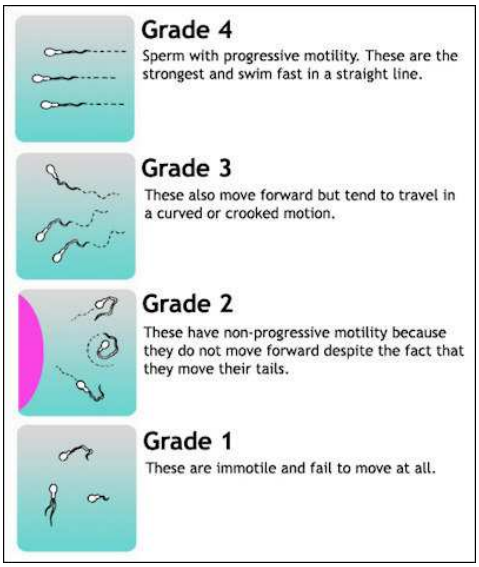
Sperm motility:

It is usually rated in two ways: the number of motile sperm as a percentage of the total, and the quality of forward progressive sperm movement i.e., how fast and how straight the sperm swims.

The degree of forward progression is a classification based on the pattern displayed by the majority of motile sperm. It ranges from zero (no movement) to 4 (excellent forward progression). Typically, we have to observe at least 50% of the sperm with good forward progression.

Microscopic evaluation of the liquefied semen may reveal agglutination of sperm. Agglutination may be head-to-head, head-to-tail, or tail-to-tail and may suggest an inflammatory or immunologic process. Sperm morphology is subject to great variation and it is unusual to see specimens that contain more than 80% normal sperm heads.

Sperm count:

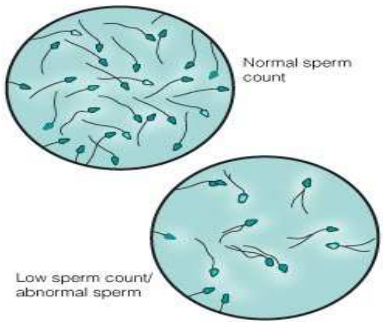


Sperm count or sperm concentration to avoid mixup, measures the concentration of sperm in a man's ejaculate, distinguished from *total sperm count*, which is the sperm count multiplied with volume Over 15 million sperm per milliliter is considered normal, according to the WHO in 2010. A lower sperm count is considered oligozoospermia. A vasectomy is considered successful if the sample isazoospermic. Some define success when rare non-motile sperm are observed (fewer than 100,000 per millilitre). Others advocate obtaining a second semen analysis to verify the counts are not increasing and others still may perform a repeat vasectomy for this situation.

The average sperm count today is around 60 million per millilitre in the Western world, having decreased by 1-2% per year from substantially higher number decades ago.

Chips for home use are emerging that can give an accurate estimation of sperm count after three samples taken on different days. Such a chip may measure the concentration of sperm in a semen sample against a control liquid filled with polystyrene beads.

5. **Treatment:**



Infertility can be treated by various methods as it is a common disorder found across the world. There are some of the theraptes listed here.

Non-surgical/ Medication Therapy:

Spinal Cord Injury (SCI) Treatments:

Many factors may predispose spinal cord injured men to infertility.

Ejaculatory dysfunction, abnormalities of sperm production, chronic infections and blockage of sperm within the male reproductive tract are all potential factors.

There is a number of different methods to obtain sperm, often combined with various forms of assisted reproductive techniques.

For example, sperm can often be obtained through vibratory stimulation to the head and shaft of the penis if the level of injury is T-12 or above. Other therapies commonly used it the rectal probe. Electro ejaculation (EEJ) or sperm harvesting along the ejaculatory path from the vas deferens, epididymis, and directly from the testis.

Electro ejaculation therapy (EEJ):

This is a very successful form of therapy for men who have normal sperm production but cannot ejaculate because of a short circuit in the nervous system. Initially used for men with spinal cord injuries, EEJ has also proven effective for loss of ejaculation in patients with other conditions such as diabetes, retroperitoneal lymph node dissection (RPLND), pelvic surgery, multiple sclerosis, or unexplained loss of orgasm. For patients with intact sensation, a pain free procedure of 30 minutes with local anesthesia at an outpatient surgery centre. EEJ is non-invasive and patients routinely return back to desk type work that day.

Electroejaculation allows the retrieval of sperm in more than 90% of patients and up to 40% of couples will achieve pregnancy with IUI.. The very powerful sperm injection form of IVF, called ICSI, gives the remaining couples a 50% chance for pregnancy. Overall, the chances for pregnancy in the informed and motivated patient are similar to those of a healthy male.

Transurethral Resection of Ejaculatory Duct:
Transurethral resection of the seminal vesicles (TURSV):
The procedure eliminates obstruction in the seminal tract or in a strategic area, improving the chances of the inflamed or chronically dilated zones to normalize.

Medication:
Hormone deficiency treatments:
If the man has a hormonal deficiency, it might be treatable with medications. These are rare cases.

Clomiphene citrate (Clomid, Serophene):

Some men with relatively mild sperm abnormalities have been treated with clomiphene citrate(tablets) in an attempt to improve the semen. Research showed that Clomid for the male sometimes can improve the sperm count or motility.
Surgery:

Varicocele ligation:

If a varicocele is found, sometimes surgery to ligate (tie off) the abnormally dilated veins is recommended. If the varicocele is of significant size (Grade II or Grade III), about two thirds of men undergoing the surgery will see improvement in the sperm quality. Pregnancy rates following surgery are in the range of 40%, but most pregnancies occur 6-9 months following surgery depending on the female's age.

If the initial sperm count and motility are in the severe male factor category, it is unlikely that this surgery will improve sperm counts enough to enable the couple to conceive without assistance.

Sperm Retrieval Techniques (MESA, PESA, TESE):

Using today's minimally invasive techniques, sperm can be obtained from men with vasectomy; failed vasectomy reversal, absence of the vas deferens, or uncorrectable blockages anywhere along the seminal tract (obstructive azoospermia). In addition, we are able to retrieve and use sperm in case of non-obstructive Azoospermia or NOA.

Sperm retrieval procedures are typically done at an outpatient surgery and last about one hour. Local anesthetic, IV sedation or general anesthesia provides complete pain control during the procedure. Patients return back to desk type work in a day or two.

Prevention of male infertility:

Most types of infertility cannot be prevented. Smoking has been linked to low sperm counts and sluggish sperm movement in men, and an increase in miscarriage in women. , Alcohol affects the fertility of both men and women trying to conceive either naturally or through infertility treatments. Alcohol is toxic to sperm; it reduces sperm counts, can interfere with sexual performance, Other useful methods include meditation, relaxation, moderate physical activity and yoga, A well-balanced diet includes carbohydrates, protein and fibre., Avoid environmental poisons and hazards such as pesticides, lead, heavy metals, toxic chemicals, and ionising radiation

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An HPLC method for the Simultaneous estimation of Risperidone and Trihexyphenidyl hydrochloride from Bulk and Dosage forms.

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Abstract

A simple, fast and precise reverse phase high performance liquid chromatographic method developed for the simultaneous determination of risperidone and trihexyphenidyl hcl in its tablets form. C_{18} phenomenex Gemini column 250mm x 4.6mm (l x d) in isocratic mode with mobile phase buffer: Acetonitrile: methanol (50:30:20) % v/v were used. The flow rate was 1 ml/min. Linearity for risperidone and trihexyphenidyl hcl were in the range of 96.96 mcg/ml – 145.44mcg/ml and 65.60 mcg/ml – 98.4 mcg/ml respectively. Amount found of risperidone and trihexyphenidyl hcl in sizodon plus was 3.034 mg/tab and 2.063 mg/tab respectively. Amount found of risperidone and trihexyphenidyl hcl in RI- plus was 3.016 mg/tab and 2.044 mg/tab respectively. Percentage recoveries obtained for risperidone and trihexyphenidyl hcl in sizodon plus were 100.87% and 102.49% respectively, percentage recoveries obtained for risperidone and trihexyphenidyl hcl in RI-plus were 100% and 101.65% respectively. The proposed method is accurate, precise, selective and rapid for the simultaneous estimation of risperidone and trihexyphenidyl hydrochloride in tablet dosage.

Key words: HPLC, Validation, Risperidone, Trihexyphenidyl hydrochloride

1. Introduction

Risperidone has exhibited good therapeutic efficacy against both positive and negative schizophrenic symptoms with low incidence of EPS. Chemically the drug is 3-[2-[4-(6- fluoro -1, 2-benzisoxazol -3-yl) -1 piperidinyl] ethyl] -6,7,8,9- tetrahydro – 2 methyl – 4 H – Pyrido [1, 2 – a] Pyrimidin – 4 – one.

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Several methods such as LC/ MS/ MS[1] , HPLC[2], LC-MS[3] have been reported in the literature. Trihexyphenidyl Hcl is used to treat all forms of Parkinsonism. This medicine may also be prescribed to control extrapyramidal disorders (except tar dive dyskinesia) due to central nervous system drugs. Chemically the drug is 1- cyclohexyl -1- phenyl -3 (1- piperidyl) propane -1- ol. Several methods such as HPLC[4], HPLTC[5], have been reported in the literature.

2. **Materials and methods**

HPLC pump isocratic LC 20 AT equipped with rheodyne injection volume 20 µl, uv – vis detector SPD – 20 A. Reference standard of risperidone and trihexyphenidyl hcl. Sizodon – plus (Sun pharmaceuticals), RI-plus (VGR bio Laboratories) were procured from market. Label claim of sizodon plus tablets is Risperidone-3mg and Trihexyphenidyl-2mg. Label claim of RI-Plus tablets is Risperidone-3mg and Trihexyphenidyl-2mg. Acetonitrile HPLC grade, water of HPLC grade, orthophosphoric acid HPLC grade, methanol HPLC grade, Trienthylamine HPLC grade reagents were used. Stationary Phase C_{18}, 5µ, phenomenex Gemini column 250mm x 4.6mm (LxD) was used.

0.3% orthophosphoric acid was prepared in water and adjusted the pH to 3.0 with triethylamine as buffer. 50ml of buffer was mixed with 30ml of Acetonitrile and 20ml of methanol to form 100ml of mobile phase, finally filtered through membrane filter 0.45mm micron degassed.

2.1. **Standard Stock Solution**

30.3mg of risperidone and 20.5mg of Trihexyphenidyl Hcl were taken in 50ml volumetric flask, dilute with little amount of mobile phase and the contents were shaken thoroughly and finally make up the volume to 50ml with the same mobile phase. 5ml of the above solution is transferred to a 25ml volumetric flask, dilute with little amount of mobile phase and the contents were shaken thoroughly and finally make up the volume to 25ml with the same mobile phase.

2.2. **Sample Solution**

10 tables were weighed and crushed to obtain a fine powder and transferred to a 50ml volumetric flask. Little amount of mobile phase was added and the contents were shaken thoroughly and finally make up the volume to 50ml. 5ml of the above solution is taken and transferred a 25ml volumetric flask. Little amount of mobile phase was added and the contents were shaken thoroughly and finally make up the volume to 25ml.

3. **Results and discussion:**

3.1. **Assay:** 20µl standard stock solution and sample solutions (n=4) were injected in to an injector of liquid chromatograph. From the peak area of risperidone and trihexyphenidyl Hcl, the amount of drugs in samples (n=4) were computed
A typical HPLC chromatogram of Risperidone and Trihexyphenidyl hydrochloride as shown in fig 1. In replicate analysis n=4 of two drugs by the proposed method the content of risperidone and trihexyphenidyl Hcl in sizodon plus were 3.034 mg / tab and 2.063mg/tab respectively. The content of risperidone and trihexyphenidyl Hcl in RI-plus were 3.016mg/tab and 2.044mg/tab respectively. The results obtained by the proposed method were close to the label claim of both the drugs indicating that the method is precise and accurate.

3.2. **Linearity Study:** In to a series of five standard measuring flask, varying amount of standard stock solution of combination of risperidone and trihexyphenidyl Hcl was taken and made up to various concentrations of 96.98, 109.08, 121.20, 133.32, 145.44 mcg/ml (80, 90, 100, 110, 120%) for risperidone and 65.60, 73.80, 82.0, 90.2, 98.4 mcg/ml (80, 90, 100, 110, 120%) for trihexyphenidyl hcl, 20µl was injected from each flask. The peak area response of the solutions were recorded at 220nm. The plot of peak area versus the respective concentrations of risperidone and trihexyphenidyl Hcl were found to be linear in the range of 96.98 – 145.44mcg/ml and 65.60 – 98.4mcg/ml respectively with coefficient of correlation (r = 0.9999) and (r = 0.9998) respectively as shown in Fig.2.

3.3. **Accuracy:** Accuracy studies were performed at 80%, 100%, 120% spiked sample. Three replicates of each concentration were performed. The mean percentage recovery of risperidone and trihexyphenidyl hcl in sizodon plus were 100.87% and 102.49% respectively. The mean percentage recovery of risperidone and trihexyphenidyl hcl in RI-plus were 100.00% and 101.65% respectively. Since there is no significant difference between the theoretical and actual, the method is shown to be accurate and selective.

3.4.**Robustness:** Robustness mobile phase alter increasing 20% buffer, the value of retention time, number of theoretical plates, tailing factor for risperidone & trihexyphenidyl hcl were 4.457,2797,1.58 & 9.730,6690,1.14 respectively. Robustness mobile phase alter increasing 20% acetonitrile, the value of retention time, number of theoretical plates, tailing factor for risperidone & trihexyphenidyl hcl were 1.580,2581,1.48 & 3.543,3897,1.55. respectively. It shows the reliability of an analysis with respect to deliberate variations in method parameters.

3.5.**System Suitability:** System suitability tests were carried out on freshly prepared standard stock solution of risperidone and trihexyphenidyl Hcl and the parameters obtained with 20µl injection volume and standard stock solution(n=6) are shown in Table 1

4. **Conclusion:**

The proposed method is simple**,** precise and accurate for the simultaneous determination of risperidone and trihexyphenidyl hydrochloride in tablet dosage. In routine Quality control or in Test Laboratories, when we have this formulation to be analyzed, the method is best suited.

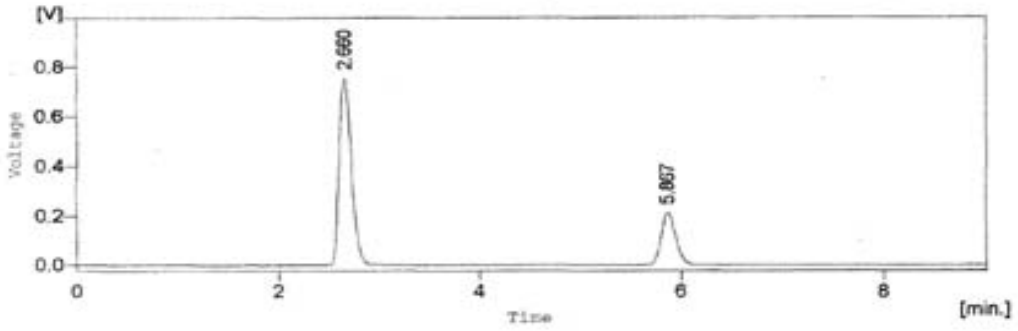

Fig. 1 Typical HPLC Chromatogram Of Risperidone & Trihexyphenidyl Hcl

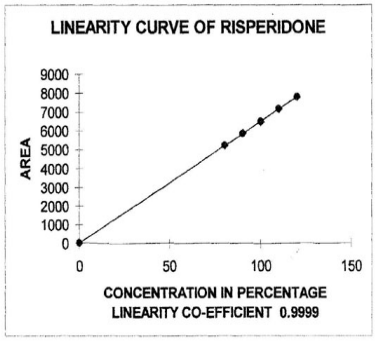

Fig .2 Typical Linearity curve of Risperidone and Trihexyphenidyl hydrochloride

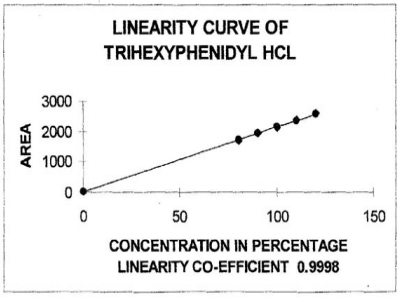

Table 1 System Suitability Parameters

Parameters	Risperidone	Trihexyphenidyl hcl
Retention time	2.685	5.912
Tailing factor	1.351	1.200
Theoretical plate	2161.83	7972.83
Peak area	6448.461	2188.988
RSD of peak area	0.31%	0.53%

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Anti-inflammatory and anti-oxidant activities of the methanolic extracts of the stalk of *Parkia biglobosa* (jacq.) Benth.

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Abstract

Oral administration of various doses (50, 100 and 250 mg/kg) of the methanol extracts of the stalk of *Parkia biglobosa* produced anti-inflammatory activities by reducing the croton oil ear inflammation, though not statistically relevant at $p<0.01$ level of significance. It also antagonised the oedema produced by carrageenin and arachidonic acid as well as the granuloma by cotton pellet in rats. The stalk also showed *in vitro* anti-oxidant activities using the DPPH. The extract is suspected to produce its anti-inflammatory activity by inhibiting both the lipo-oxygenase and cyclo-oxygenase pathways of the arachidonic acid metabolism.

Key words: Anti-inflammatory, Anti-oxidant, *Parkia biglobosa*

1. Introduction

Parkia biglobosa, a Fabaceae, commonly known as 'African locust bean', is a plant used extensively in West Africa for timber, food and medicine [1]. A decoction of the bark, root and leaves is used in treating toothaches, leprosy, eye sores, hypertension and fevers [2]. *P. biglobosa* pulped bark is used along with lemon for wound and ulcers [3]. It is also used against bronchitis, pneumonia, ulcers, bilharzias, malaria, diarrhoea, violent colic, venereal diseases, sterility, rickets, oedema, haemorrhoids and toothaches [4]. The pulp is used as a diuretic and mild purgative [5]. The fermented seeds of *P. biglobosa* are used in all parts of Nigeria and indeed the West Coast of Africa for seasoning traditional soups [2]. Previous pharmacological studies [6] showed the stem bark as potent anti-snake venom.

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P. biglobosa have been shown to contain glycosides, tannins, slight presence of alkaloids, steroids were negligible. However, there was complete absence of saponins and anthraquinones [7].

A decoction of the stalk is traditionally used in the treatment of arthritic pains. This present work therefore, was undertaken to investigate anti-inflammatory and anti-oxidant effects of the methanol stalk extracts of *P. biglobosa.*

2. **Materials and Methods**

2.1. **Plant Material**

The stalks of *P. biglobosa* were collected from Nsukka Urban Area, Enugu State, Nigeria in November, 2009 and plant parts were identified and authenticated by Mr. A.O. Ozioko, a taxonomist with the International Centre for Ethnomedicine and Drug Development, Nsukka. A voucher specimen with number MOUAU/CVM/VPP/HB503 was deposited in the herbarium of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike for reference.

2.2. **Preparation of the extract**

The dried plants were pulverized into coarse powder and were extracted in 80% methanol for 48 hours with intermittent shaking. Thereafter, filtration was done using filter papers and funnel into an already weighed beaker. The solvent was allowed to evaporate in a rotary evaporator at 40^{o}C and water using a lyophilizer.

2.3. **Animals**

Mature out-bred Albino rats of both sexes weighing 150–175g were purchased from the Laboratory Animal Facility of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka and used. They were kept in clean stainless steel wire mesh cages, maintained at normal temperature and natural daylight/night conditions. They were allowed free access to standard commercial pelleted feed and clean drinking water. Ethical conditions as stipulated by Ward and Elsea [8] in the conducts of experiments with life animals were adhered to strictly. The study protocol was approved by the University's ethical committee.

3. **Anti-inflammatory activity**

3.1**.** *Cotton pellet-induced granuloma*

Five groups of six rats each were used. Two cotton pellets weighing 10 mg were autoclaved and implanted subcutaneously into both sides of the interscapular region of each anaesthetized rat with the incision closed by interrupted sutures after expelling air from the tunnels [9].

P.biglobosa at doses of 50, 100 and 250 mg/kg, ibuprofen 100 mg/kg and distilled water were administered through the intraperitoneal route starting from the day of pellet implantation. They were treated daily for 7 days. On the 8th day, animals were sacrificed with ether; the pellets together with the granuloma tissues were carefully removed, dried in the oven at 60°C, weighed and compared with the control.

3.2. *Carrageenin induced rat paw oedema*

Oedema was induced by the methods of Winter *et al* [10]. Six rats of either sex were divided into five groups and treated with 50, 100 and 250 mg/kg of the extracts, ibuprofen 100 mg/kg and the vehicle, distilled water (1 ml/kg) respectively, 30 minutes prior to an injection of Carrageenin (0.1ml/100g from a 10mg/ml solution) into the planter aponeurosis of the right hind paw of the rats. The left hind paw served as control receiving 0.1 ml of saline. The paw volume was measured plethysmographically 4 h after carrageenin injection.

3.3. *Croton oil-induced ear inflammation*

Croton oil irritant solution (0.1ml) was applied externally to the outer surface of the right ear of test rats according to the methods of Brooks *et al* [11]. Thirty rats were employed, divided into 5 groups containing 6 animals each. Group 1 (negative control) received 0.1mg/10g of normal saline intraperitoneally; group 2 received 100mg/kg of Ibuprofen, i.p.; groups 3, 4 and 5 received 50, 100 and 250 mg/kg, i.p. respectively, of the extracts (PB), 30 minutes prior to croton oil application. The rats were sacrificed with ether after 4 h and 7mm punches were made in the ear with a cork borer. Each ear disc was weighed and compared with the control.

3.4. *Arachidonic acid-induced paw oedema*

The methods of DiMartino *et al* [12] were employed in the experiment. Thirty six male albino rats grouped into 6 of 6 animals each and treated intraperitoneally with the vehicle, normal saline (0.1 ml/10g), a double blocker, phenidone (100 mg/kg), indomethacin (10 mg/kg) and the extracts (50, 100 and 250 mg/kg). Paw oedema was induced in the right plantar surface of the hind paw, 30 minutes post-treatment by a single injection of 0.1 ml of 0.5% arachidonic acid in 0.2M carbonate buffer (pH 8.4). The hind paw volume was measured 1 h after arachidonic acid injection.

4. **Anti-oxidant activity.**

DPPH photometric assay

The free radical scavenging activity of the extract was analyzed by the 1, 1–diphenyl-2-picryl-hydrazyl (DPPH) assay described by Iwalewa *et al* [13].

Two (2) ml of the test extract, at concentrations of 10 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml each was mixed with 1 ml of 0.5 mM DPPH (in methanol). The absorbance of the resulting solution was read at 517 nm after 30 minutes of incubation in the dark at room temperature using a spectrophotometer.

One (1) ml of 1000 µM ascorbic acid in 1 ml of DPPH was used as reference standard antioxidant while a blank of 1ml methanol plus 2 ml of extract was ran with each assay. All determinations were carried out in triplicate. The same procedure was repeated using control sample (DPPH without extracts). Mean values were obtained and used for the following calculation:

% Antioxidant Activity [AA] = [{(Abs $_{sample}$ – Abs $_{blank}$) **X** 100}/ Abs $_{control}$].

5. **Data analysis**

Data obtained were presented as mean ± SEM and analysed using one-way analysis of variance (ANOVA) and post-hoc comparisons were carried out using Dunnett's *t*-test. Values of $p<0.05$ were considered significant in the study.

6. **Results and Discussion**

The yield of the extract obtained was 14.5%. Intraperitoneal administration of the methanolic extracts of *Parkia biglobosa* stalk significantly antagonized the formation of croton pellet granuloma (Table 1) in a dose-dependent manner.

The extract also showed a dose-dependent inhibition of the croton oil ear inflammation in test animals (Table 3). There was also appreciable inhibition of carrageenin-induced rat paw oedema compared with controls. The difference observed between the 100 and 250 mg/kg was not statistically relevant at $p<0.05$ level of significance (Table 2).

The extracts of PB further inhibited the arachidonic acid induced paw oedema in a dose-dependent manner comparable to the dual-blocker, phenidone (Table 4). Thus, suggesting that the observed anti-inflammatory activities may be produced by the inhibition of the lipo-oxygenase pathways, the cyclo-oxygenase pathways or both which are involved in metabolism of arachidonic acid.

Findings from this study unearths the potential of the stalk of *P. biglobosa* as anti-oxidant (Figure 1) which could be exploited in drug development urgently needed to challenge free radicals in biological systems and consequently prevent the body from reactive oxygen species (ROS) originated ailments, which may include among others cancer, arthritis, diabetes.

7. **Conclusion**

Based on these results, we concluded that administration of the stalk of *P. biglobosa* either orally or parenterally results in anti-inflammatory activity. The *in vitro* anti-oxidant activity of the extract gives credence to the folkloric use of the plant as an agent against inflammatory conditions such as arthritis.

Table 1. Effect of *P. biglobosa* stalk extracts on Cotton pellet-induced granuloma

Drug	**No. of animals**	**Dose (p. o.)**	**Weight of granuloma (mg)**
Normal saline	6	0.1ml/10g	70.0 ± 4.0
Ibuprofen	6	100mg/kg	48.0 ± 2.0*
PB	6	50mg/kg	48.0 ± 2.0*
PB	6	100mg/kg	43.0 ± 1.0*
PB	6	250mg/kg	35.0 ± 1.0*

Values are means ± SEM. *p<0.05

Table 2. Effect of *P. biglobosa* stalk extracts on Carrageenin-induced rat paw oedema

Drug	**No. of animals**	**Dose (p. o.)**	**Paw volume (ml)**
Normal saline	6	0.1ml/10g	0.61 ± 0.006
Ibuprofen	6	100mg/kg	0.21 ± 0.02*
PB	6	50mg/kg	0.29 ± 0.005*
PB	6	100mg/kg	0.32 ± 0.005
PB	6	250mg/kg	0.33 ± 0.005

Values are means ± SEM. *p<0.05

Table 3. Effect of *P. biglobosa* stalk extracts on Croton oil-induced ear inflammation

Drug	No. of animals	Dose (p. o.)	Weight of ear disc (mg)
Normal saline	6	0.1ml/10g	13.0 ± 5.0
Ibuprofen	6	100mg/kg	11.0 ± 2.0*
PB	6	50mg/kg	12.0 ± 5.0*
PB	6	100mg/kg	11.0 ± 4.0*
PB	6	250mg/kg	10.0 ± 4.0*

Values are means ± SEM. *p<0.05

Table 4. Effect of *P. biglobosa* stalk extracts on Arachidonic acid induced paw oedema

Drug	No.	Dose (i.p.)	Paw volume difference (Mean ± SEM) ml	% inhibition as compared to control
Normal Saline	6	0.1ml/10g	4.22 ± 0.25	-
Indomethacin	6	10 mg/kg	2.51 ± 0.39*	40.52%
Phenidone	6	100 mg/kg	0.94 ± 0.15*	77.73%
PB	6	50 mg/kg	1.85 ± 0.34*	56.16%
"	6	100 mg/kg	1.01 ± 0.25*	76.07%
"	6	200 mg/kg	0.64 ± 0.12*	84.83%

Values are means ± SEM. *p<0.01 vs. group 2.

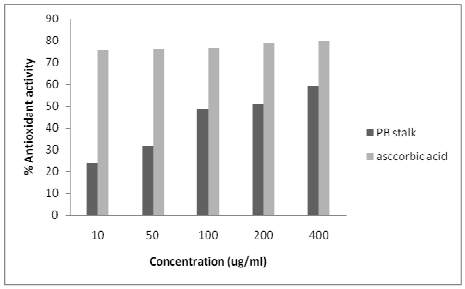

Figure 1. The antioxidant activities of methanol stalk extracts of *P. biglobosa* using the DPPH assay.

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WOUND HEALING PROPERTIES OF *CLEOME VISCOSA* LINN

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Abstract

The leaves and whole plant of *Cleome viscosa* are used as a folk remedy to cure the wounds, ulcers, inflammations and skin infections. The present investigation was undertaken to evaluate the wound healing property of the leaves and whole plant of *Cleome viscosa* on experimentally induced excision wound model in rats. The studies on the wound healing models revealed that, the methanolic extract of *Cleome viscosa* possess significant wound healing activity,

***Key words*:** *Cleome viscosa;* wound healing property; excision wound model; wound; skin infections

1. Introduction

Cleoma viscosa (Family: Cleomaceae) is an annual erect, branched, viscid pubescent herb in 30-90cm height with 3-7 foliate leaves, white, yellow, pink flowers, stems grooved, densely clothed with glandular and simple hairs found in waste grounds and grassy places. The natives and traditional healers of India called this plant as 'Hul hul'. [1,2].Traditionally the leaves, bark, root and seeds of the plants of Cleome genus are used as stimulant, ant scorbutic, anthelmintic, rubifacient, vesicant, carminative, stomachic, laxative, diuretic, anti inflammatory, anti tumour, antiseptic, anti leprosy[1,2,3,4]. The plant is good for malarial fevers and useful in blood diseases, uterine complaints. The leaves are also used in for wounds and ulcers. *Cleome viscose* leaves and young shoots used to cook like a vegetable, which is having sharp mustard like flavour. The pungent seeds and seed pods can be used as a mustard substitute in curries [6-7].

The analgesic, anti microbial, anti diarrhoeal, anti pyretic, hepatoprotective, anti hyper lipidemic and anti ulcer activities of the aerial parts has been reported [8-17]. The popular use of the whole plant and leaves refers mainly to its antiseptic, anti inflammatory activity and wound healing.

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As there is no scientific data available to substantiate the traditional use of this plant for healing wounds, in the present study, we investigated the wound healing activity of the methanolic extracts of *Cleome viscosa* leaves and whole plant in experimental animal models.

2. **Materials and methods**

2.1. *Plant material and extraction*

Cleome viscosa plant is a common weed throughout Andhra Pradesh. The *Cleome viscosa* plant material collected from local areas of Warangal, A.P. Its parts were authenticated by Prof. S.V.Raju, Taxonomist, Department of Botany, Kakatiya University, Warangal, A.P, India. A voucher specimen (CV-028) was maintained in the Department of Pharmacognosy, Vaagdevi college of pharmacy, Hanamkonda.

The fresh air- dried, powdered crude drugs obtained from the *Cleome viscosa* leaves and whole plant and were extracted with methanol solvent by maceration process at room temperature for 7 days in conical flask with occasional stirring and shaking. The methanolic extracts were dried and incorporated in to simple ointment (5 % w/w) base. Preliminary phytochemical analysis was carried out for the extracts according to the standard procedures [8, 10, 18, 19].

2.2**.** *Experimental animals*

Albino rats of either sex (130–180gm) were procured from Mahaveera Enterprises, Hyderabad under standard laboratory condition (25 ± 2°C temperature, 55± 5% relative humidity, and 12 hrs light and dark cycles for about 7 days prior to dosing. The animals were housed three per cage of same sex in polypropylene cages provided with bedding of paddy. Pellet chew feed standard diet under good management conditions and water *ad libitum* was provided to the animals.

3. **Acute-toxicity studies**

Healthy adult Wistar albino rats of either sex, starved overnight, were divided into groups (n=6) and were orally fed with increased dose of ethanol extracts. Total ethanol extracts administered orally in doses of up to 2g/Kg, did not produce any sign of toxicity and mortality in rats when observed for 7 days after administration [21].

4. **Wound healing activity by excision model**

Plan of work

Adult wistar albino rats (130-180 gm) of either sex were used for evaluation of wound healing activity. The animals were divided into 4 groups.

Group I: Received 2% sodium alginate
Group II: Received Neosporin ointment (0.2%)
Group III: Received 5% Whole plant methanolic extract ointment (WPME)
Group IV: Received 5% Leaf methanolic extract ointment (LME)
The hairs were removed from the dorsal thoracic region of the rats using depilitor and Veet hair removing cream. A full thickness excision wound of circular area of 500mm^2 and 2mm in depth was created along the markings under mild anaesthesia**.** Six animals each served as control and treated group; simple ointment and ointment containing the extracts were applied everyday topically from 0 to 22 day post wounding or number of days required for falling of the escher without any residual raw wound gave the period of epithelization, starting from the day of the excision.. Changes in wound area were calculated giving an indication of the rate of wound contraction. The areas of the wounds were measured by tracing the wounds on to a graph paper on the day of wounding and subsequently on 4th, 8th, 12th, 16th and 22nd day post wounding. The number of days required for falling of the scar without any residual raw wound, gave the period of epithelization. The observations of the percentage wound contraction were made on 4th, 8th, 12th, 16th and 22nd day post wounding days. All the values were statistically analyzed by unpaired student t test comparing with control [20, 22].

5. **Results and discussion**

Wound Healing activity study

In the acute oral toxicity studies, no mortality and no macroscopically organ abnormality/damage were observed. Acute toxicity studies showed that *Cleome viscosa* extracts were safe up to maximum dose of 2g/Kg body weight of the animal.

The animals treated with methanolic extracts of *Cleome viscosa* showed a significant wound healing activity as evidenced by the reduction in the number of days required ,for falling of the escher and the wound contraction compared to the standard. The changes in wound area were measured at fixed time intervals, viz 4th, 8th, 12th, 16th and 22nd days post wounding. Period of epithelization was 22nd days for the treatment groups, whereas it was 30 days for control group animals. The wound healing results are showed in the Table.1.
The results showed that the plant *Cleome viscosa* possessed effective wound healing activity as compared to control group, there by justifying its use in the indigenous system of medicine.

Table 1.Wound healing activity of ointment of WPME and LME of *Cleome viscosa* Linn.

GROUPS	Wound area (mm^2) ± SEM and (% of wound contraction) post wounding days					Epithelization in days ± SEM
	0 day	4th day	8th day	12th day	16th day	
CONTROL	79.02 ± 5.75 (0%)	70.06 ± 8.18 (11%)	58.53 ± 3.83 (25%)	50.6 ± 4.2 (35%)	40.5 ± 0.11 (49%)	35.06±0.46
Standard (Neosporin)	79.02 ± 5.75 (0%)	56.91 ± o.41 (27%)	22.44 ± 4.97* (71%)	2.01 ± 1.57* (97%)	0* (100%)	16.50±0.23
Whole plant Methanol extract (WPME)	81.5 ± 4.86 (0%)	54.17 ± 7.6 (33%)	22.99 ± 6.34* (72%)	2.63 ± 1.56* (97%)	0* (100%)	16.50±0.23
Leaf Methanol extract (LME)	84.77 ± 8.08 (0%)	57.43 ± 5.74 (32%)	32.18 ± 4.37* (62%)	8.01 ± 1.59* (90%)	2.64 ±1.56* (97%)	18.83±0.16

Student t-test. * -> $P < 0.05$

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Antibacterial evaluation of the methanolic extract of *Passiflora edulis*

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Abstract

In the present study methanolic from the leaves of *Passiflora edulis* for finding potential antibacterial activity. Which were compared with the standard ciprofloxacin 5µg/disc. The invitro antibacterial study efficacy of the methanolic extract was tested against Gram positive bacteria viz., *Staphylococcus aureus* (NCL 2079), *Staphylococcus faecalis* (NCL 2080), *Bacillus subtilis* (NCL 2063) and Gram negative bacteria viz., *E.coli* (NCL 2065), *Proteus vulgaris* (NCL 2027), *Salmonella typhi* (NCL 2023) by disc diffusion method. The methanolic extract showed promising activity against *Bacillus subtilis* and *E.coli*.

***Key words*:** *Passiflora edulis*, Ciprofloxacin, Antibacterial, and Methanolic extract.

1. Introduction

The search for antibacterial agent with new mode of actions will always remain an important and challenging task.[1] To a bacterium, the human body is a collection of environmental niches that provide the warmth, moisture and food necessary for organism to grow. The bacteria have acquired genetic traits that enable them to enter the environment, remain in a niche, gain access to food sources, and escape clearance by host immune and non-immune protective responses unfortunately many of the mechanisms that bacteria use to maintain their niche and the bi-products of bacterial growth are incompatible with system of the human host. Many of these genetic traits are virulence factors, which enhance the ability of bacterium to cause disease.[2] *Passiflora edulis* Sims (passion fruit, purple granadilla) is wild species belonging to the family Passifloraceae.

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The plant is a shallow-rooted, woody, perennial, tendril climbers. The alternate, evergreen leaves, deeply 3-lobed when mature, are finely toothed, 3 to 8 in (7.5-20 cm) long, deep-green and glossy above, paler and dull beneath, and, like the young stems and tendrils, tinged with red or purple, especially in the yellow form. A single, fragrant flower, 2 to 3 in (5-7.5 cm) wide, is borne at each node on the new growth[3].

Passiflora edulis reported to possess cytotoxic, antioxidant activity,[4] anti-inflammatory activity,[5] comparative biological activity,[6] neuro pharmacological activity,[7] healing of colonic anastomosis in rats,[8] healing process of gastric suture,[9] antifungal,[10] antihypertensive[11] and it is safe herbal drug which contains the constituents like a new glycoside passiflorin, ionone-I, ionone-II, megastigma-5,8-dien-4-1, megastigma-5,8(Z)-diene-4-1, 4,4a-Epoxy-4, 4a-dihydroedulan, 3-hydroxyedulan, Edulan-I, Edulan-II, passifloric acid methyl ester.[12] The leaves are simple, 3 lobed, ovate, palmate, pinnate, length is 4 to 8 inches and colour is green.

2. **Experimental**

2.1. *Plant Material*

The fresh leaves of plant specimens were collected from Nilgri Hills in Cunoor and it was authenticated [No. BSI/SC/5/23/06-07/Tech.17] as *Passiflora edulis* Sims Family: Passifloraceae in Botanical Survey of India, Tamilnadu Agricultural University, Coimbatore. Tamilnadu, India.

2.2. *Preparation of leaf extract*

The dried leaf powder of *Passiflora edulis* were extracted with methanol[7] by using Soxhlet apparatus for 48 hrs and it was concentrated *in vacuum* .

2.3. *Preliminary phytochemical studies:*

Preliminary phytochemical studies proved the presence of flavonoids in the methanolic extract of *P.edulis* leaves[7].

3. **Antimicrobial activity:**

Antimicrobial activity for the methanolic extract of *Passiflora edulis* were tested for the antimicrobial effect against bacterial strains.[13-16] The inoculums for the experiment were prepared fresh in Mueller Hinton broth from preserved frozen slants. It was incubated at 37°C for 18-24 hours and used after standardization. Mueller-Hinton agar plates were prepared marked and inoculated with Gram positive and Gram negative bacteria by Disc diffusion Technique.[17] The test microorganisms are Gram positive: *Staphylococcus aureus, Staphylococcus faecalis and Bacillus subtilis;* Gram negative: *E.coli, Proteus vulgaris and Salmonella typhi.* Were obtained from National Chemical Laboratory (NCL) Pune and maintained by periodical sub culturing on Nutrient agar medium for bacteria. The effect produced by the methanolic extract 200µg/disc and isolated pure compound 20µg/disc was compared with the effect produced by the positive control (Reference standard Ciprofloxacin 5µg/disc). (Table No: 1)

Disc diffusion assay

The anti-microbial activity of *Passiflora edulis* methanolic leaf extract against microorganisms examined in the present study and their potency were assessed by the presence and absence of zone of inhibition. The percentage of zone of inhibition was calculated by using following formula.

$$(100 - CT_D - S_D / T_D) \times 100$$

CT_D – Calculated test dose; S_D – Standard dose; T_D – Test dose

4. **Results and discussion**

The results revels that methanolic extract of *Passiflora edulis* were significantly effective against Gram positive bacteria *Bacillus subtilis* and Gram negative bacteria *E.coli*. Also methanolic extract was suggestively against *Staphylococcus aureus* and Gram negative bacteria *Salmonella typhi* when compared with standard ciprofloxacin under similar conditions.

5. **Conclusion**

In conclusion the methanolic extract showed promising activity when compared with the standard ciprofloxacin against both Gram positive and Gram negative bacteria

Table: 1 Antibacterial activity of methanolic extract of the leaves of *Passiflora edulis*

S.No	Microorganisms	Zone of Inhibition in Mm and % Samples			
		A	STD	A%	STD%
01.	*Staphylococcus aureus* (NCL 2079)	10±1.03	30±0.78	0.83%	100%
02.	*Staphylococcus faecalis* (NCL 2080)	15±1.24	32±0.88	1.17%	100%
03.	*Bacillus subtilis* (NCL 2063)	18±0.88	35±1.12	1.28%	100%
04.	*E.coli* (NCL 2065)	25±1.54*	38±0.76**	1.64%	100%
05.	*Proteus vulgaris* (NCL 2027)	8±1.36	30±0.64	0.66%	100%
06.	*Salmonella typhi* (NCL 2023)	15±1.12	32±1.62	1.17%	100%

n=3 A - Methanolic extract**;** STD - Standard Drug Ciprofloxacin. student t test , * p value<0.05, ** p value<0.01

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Comparative diuretic activity of seed and Fruit wall extract of *Solanum torvum*

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Abstract

The Pharmacognostical and phytochemical examinations of methanol extracts of seeds and fruit wall of *Solanum torvum* were conducted and their diuretic activity was evaluated in albino rats. The administration of methanol extracts of seed and fruit wall of *Solanum torvum* by oral route at doses; 150,300 and 450 mg/kg body weight. After 5 hr the volume of urine is measured . Results revealed that the fruit wall methanol extract showed significant diuretic activity comparative to seed methanol extract and concentrations of potassium & sodium salts in urine as compared to standard drug Furosemide.

Keywords: *Solanum torvum*, *Methanol*, Furosemide, diuretic activity.

1. Introduction

Diuretics are the drugs capable of increasing the rate of urine flow and sodium excretion and are used to adjust the volume and composition of body fluids in a Varity of clinical situations, including hypertension, heart failure, renal failure, nephritic syndrome and cirrhosis. These drugs act on the kidney and are able to increase the volume of urine excretion. The urine output increases after administration of diuretic drugs like furosemide [1]. *Solanum torvum (Solanaceae)* popularly know as Sundaikai, Kodusonde in India, is used in the treatment improving the eyesight and treatment of spleen and liver enlargement, anti microbial agent and the plant is considered sedative, diuretic and digestive. [2, 3].

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Since the diuretic activity of this plant has not been scientifically evaluated, the present study was undertaken to investigate the effect of methanolic extracts of seed and fruit wall of *Solanum torvum* for its diuretic activity with their electrolyte excretion.

2. Materials and methods

2.1. *Plant material*

The Fruits of *Solanum torvum*. (Solanaceae) were collected in September and October 2007 from Thirumala Thirupathi (A.P), India. The plant parts are identified by Dr. Madhavashetty, Taxanomist, Dept of Botany, S.V University and Thirupathi, India and authenticated by comparing with the voucher specimen.

2.2. *Extraction*

The Seed, fruit wall are separated, dried and powdered and macerated with methanol for 2 days to afford a greenish brown semisolid mass (Seed methanol extract; yield: 6.76% w/w on dried wt, Fruit wall methanol extract; yield:7.12% w/w on dried wt,).

2.3. *Preliminary phytochemical studies*

Preliminary qualitative phytochemical analysis of Seed methanol extract and Fruit wall methanol extract indicated the presence of spirostanol glycosides, isoflavanoids, alkaloids, tannins and carbohydrates.

2.4. Animals

Wistar rats of either sex, weighing 180-240 g purchased from NIN, Hyderabad were used. They were housed in standard environmental conditions of temperature, humidity, light and provided with standard rodent food and water *ad libitum*

3. **Diuretic Activity**

The Wistar rats were divided into eight groups of six animals each. Group I served as control and received normal saline orally. Group II served as positive control and received Furosemide (20 mg/kg). Group III, IV and V received Seed methanolic extract, orally at a dose of 150, 300 and 450 mg/kg respectively. Group VI, VII and VIII received Fruit wall methanolic extract, orally at a dose of 150, 300 and 450 mg/kg respectively.

Immediately after administration, the animals were placed in metabolic cages specially designed to separate urine and faeces at room temperature of 25± 0.5^{o}.

The observed parameters were total volume Na^+, K^+ and Cl^- excreted in the urine. The concentration of $Na^+$and K^+ were measured by flame photometer and Cl^- concentration was estimated by titration with silver nitrate with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate as an indicator. [6, 7] Data are presented as Mean ± SEM.

4. **Statistical analysis**

Statistically, the values were analyzed with the analysis of variance (one way ANOVA) method to determine the significance of difference within the experimental groups.

5. **Results and Discussion**

The data showed that, the fruit wall methanolic extracts of *Solanum torvum* produced significant diuretic activity ,evidenced by the increased excretion of sodium and potassium salts, comparable to the standard drug, furosemide. Hence we concluded that the fruit wall methanolic extracts of *Solanum torvum* showed effective diuretic activity by increasing the total urine output and increased excretion of sodium and potassium salts. Further research is warranted to evaluate the exact mechanism and chemical compounds responsible for this activity.

Table-1Diuretic activity of seed and fruit wall extracts of *Solanum torvum*

Name of the drug/extracts	Dose (mg/kg)	volume of urine in ml (Mean ± SEM) After 5 hrs	Electrolyte excretion Na^+	K^+	Cl^-
Control	-	0.6±0.04	64	12.2	52
Standard (furosemide)	20	3.2±0.44	102	13.1	112
Seed methanol	150	0.9±0.13	72	12.2	76
	300	1.2±0.06	56	12.5	64
	450	1.4±0.13	84	13.1	81
FruitWall methanol	150	1.7±0.04	98	12.5	87
	300	2.0±0.02	114	12.8	104
	450	2.3±0.13	125	13.0	101

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MICROWAVE ASSISTED ISOLATION OF MUCILAGE FROM THE FRUITS OF *ABELMOSCHUS ESCULENTUS*

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Abstract.

A microwave assisted extraction technique was developed to optimize the extraction of mucilage from the fruits of *Abelmoschus esculentus* plant. The plant has been extracted by conventional and microwave assisted methods for the isolation of mucilage. Microwave extraction at 160 W intensity and 40 min heating duration increase 11.55% in the yield of mucilage when compared to 1 h conventional heating method. The products obtained by both the methods were of similar nature chemically. The developed microwave procedure can be used successfully in commercial and routine laboratory isolation of mucilage.

Key Words: Microwave, Mucilage, *Abelmoschus esculentus.*

1. **Introduction**

In the past few years, microwave heating has been found to be a convenient source of energy not only in kitchen, but also in chemical laboratories [1]. Many of the basic principles of green chemistry are best suited for microwave processes [2]. It is one of the simple, fast, clean, eco-friendly and efficient method. It is economic in saving energy, fuel and electricity. A very short response time and better yields of the products are the main advantages of microwave heating. Commercial microwave ovens are used as a source of energy in chemical laboratories for efficient heating of water, moisture analysis, wet ashing of biological and geological materials, waste material management, sterilization of pharmaceutical preparations, inactivation of enzymes in food products, etc.

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In the past few years, a large number of reports have appeared on the use of this technique in acceleration of organic reaction for rapid and green synthetic procedures[1]. Extraction is one of the most crucial point in the analytical chain in the effort of achieving a complete recovery of target compounds. Recently, microwave energy is being used for extraction of phytoconstituents from plants [3,4].

Microwave extraction follows the same principle as maceration or percolation, but the speed of breaking up of plant cells and plant tissues is much higher. This reduced processing time is an economic advantage and also there is less risk of decomposition or disintegration and oxidation of the valuable plant constituents. Microwave assisted extraction methods require shorter time, less solvents, higher extraction rate and better products with lower costs [4]. The microwave consists of a number of radiation chambers in order to manipulate the required energy. The energy requirement can be controlled much better than with conventional thermal energy. A great deal of work is required to standardize experimental procedures using this technique.
Today, the whole world is turning towards finding suitable alternatives for the synthetic compounds used in pharmaceutical industries from natural sources [2].

The synthetic polymers used as excipients suffer from many disadvantages such as high cost, toxicity, non- biodegradability and environmental pollution caused during their synthesis[5]. Natural polymers like mucilages and pectins are easy to isolate, purify and are non-toxic and biocompatible. They are biodegradable and will not cause environmental pollution [5]. They are found as common ingredients in cosmetic, food and non-food industries. They are used as binding, thickening, emulsifying, suspending and stabilizing agents in pharmaceutical industries and used as matrices for sustained release of drugs, because of their higher water swellability, non-toxicity, low cost and free availability [5].

Conventionally, mucilages are isolated by heating for 1 h, in aqueous solvents [5,6]. In order to reduce the duration involved in their extraction and study the yield, mucilage from the fruits of *Abelmoschus esculentus* plant was isolated in the present study using microwave method.

2. **Materials and methods**

2.1. **Collection and Authentication:**

The fruits of *Abelmoschus esculentus* (Malvaceae) plant were used for the isolation of mucilage. These materials were purchased from the local market of Bardoli, Gujarat and authenticated by Botanist from Science College Bardoli. The voucher specimen was kept in the college museum (Voucher No. VBTCP-011).

2.2. **Isolation of Mucilage by Conventional Procedure:**

Abelmoschus esculentus fruits (5 g) were powdered for 5 min in a mechanical blender and soaked in distilled water (150 ml) for 24 h in a RB flask. It was boiled for 1 h under reflux with occasional stirring and kept aside for 2 h for the release of mucilage into water. The material was filtered through a muslin bag and hot distilled water (25 ml) was added through the sides of the marc and squeezed well in order to remove the mucilage completely.

Equal volume of ethanol was added to the filtrate to precipitate the mucilage and kept inside a refrigerator for one day for effective settling. It was filtered and dried completely in an incubator at 37º C, powdered and weighed. It was subjected to chemical tests to confirm its identity [6,7].

2.3. **Isolation of Mucilage by Microwave Procedure:**

Abelmoschus esculentus fruits (5 g) were powdered in a mechanical blender for 5 min and soaked in distilled water (150 ml) for 24 h in a 1000 ml beaker. It was kept in a microwave oven (LG Grill Intello wave System, Model No. MG-2381LE) along with a glass tube inside to prevent bumping. It was subjected to microwave irradiation at 800 W intensity for 3 min. The beaker was removed from the oven and kept aside for 2 h for the release of mucilage into water. It was processed in a similar way as explained in the conventional procedure, weighed and chemical tests were carried out.The experiment was repeated several times using various intensities and different durations as shown in Table 1. In each case, the yield was calculated.

3**. Results and discussion**

Using *Abelmoschus esculentus* fruits for the isolation of mucilage, microwave standardization was carried out at several intensities and durations (Tables 1). Using 160 W intensity and 40 min heating duration, 11.55 % increase in the yield of mucilage compared to the conventional heating method.

The mucilage isolated by both the methods was found to be identical in nature and gave positive results for all the chemical tests performed. The chemical tests that were conducted are: Ruthenium red test and Molisch test. Thus, the selected plant source produced high yield of mucilage in shorter duration by the developed microwave method when compared to conventional heating.

In recent years, modern techniques are effectively being used for the extraction and isolation of phytoconstituents. Ways to minimize the consumption of energy and developing efficient isolation and purification processes is of utmost global importance today. Plant mucilages are found as common ingredients in cosmetic, pharmaceutical, food and non-food industries due to their low cost compared to the synthetic polymers [8]. In view of the rising costs and fluctuations in availability of the synthetic polymers, scientists are engaged in finding suitable alternatives to these [9]. Such an effort would be welcomed both locally and internationally. In India, only few units are manufacturing mucilages on commercial scale. USA, Switzerland and other European countries are producing these in large scale. Most of the Indian needs are mainly met by import. The conventional isolation of mucilages from plants consumes more time and gives low yields. Hence, the cost of production of these is increasing.

In the present study, mucilage was isolated in high yields and in lesser durations by the developed microwave method when compared to the conventional heating. Same quantities of raw materials were used for both the methods.

The developed microwave methods save 20 min heating duration, for the isolation of mucilage. The speed of breaking up of plant cells and plant tissues is much higher under microwave conditions [4]. The present study also proves the same.

4. **Conclusion**

In the present study, a microwave assisted method for rapid extraction of mucilage from *Abelmoschus esculentus* fruits has been optimized. The developed method improved the yield of mucilage from the selected source. In conclusion, the developed microwave procedure can be used successfully in commercial and routine laboratory isolation of mucilage.

Table 1: Standardization of microwave method for the isolation of mucilage from *Abelmoschus esculentus* fruit.

	Conventional Method			**Microwave Method**		
Sr. No.	**Duration**	**Yield (g)**	**Intensity (W)**	**Duration (min)**	**Yield (g)**	**Percent increase in yield***
1	1h	0.476	800	3	0.273	--
2			640	5	0.180	--
3			480	10	0.309	--
			320	30	0.099	
5			320	20	0.349	--
6			160	30	0.195	--
7			160	40	0.531	11.55
8			160	60	0.389	--

*In comparison to conventional method

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The effects of *Acanthospermum hispidum* extract on the Antibacterial activity of Amoxicillin and Ciprofloxacin

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Abstract

The effect of the sub-inhibitory concentrations of *Acanthospermum hispidum* extract on the activities of amoxicillin and ciprofloxacin was assessed by the Kirby-Bauer disc diffusion method. The extract demonstrated antibacterial activity against *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Salmonella thyphi, Proteus vulgaris* and *Pseudomonas aeruginosa* with MICs ranging between 11 and 53 mg/ml. A 5 mg/ml extract enhanced significantly ($p<0.05$) ciprofloxacin activity (up to 38 folds) against all the test bacteria except *Ps. aeruginosa*. Amoxicillin activity was also potentiated significantly ($p<0.05$) against *Staph. aureus* (9 fold) and *B. Subtilis* (12 fold). *A. hispidum* appears to contain phytoconstituents that may be useful adjuvant for antibiotic formulations.

Key words: Sub-inhibitory concentration, antibacterial activity, amoxicillin, ciprofloxacin, *A. hispidum.*

1. Introduction

Antibiotics, once considered the universal answer to infectious disease, are now known to have a limited effective life span. Disease causing microorganisms that were once thought to have been controlled by antibiotics are returning in new forms resistant to antibiotic therapy [1]. Incidents of epidemics due to such drug resistant microorganisms are now a common global problem posing enormous public health concerns. While chemical modifications could be significant in antibiotic resistance, exclusion from the cell of unaltered antibiotic represents the primary strategy in denying the antibiotic access to its targets and this is believed to enhance resistance even in cases where modification is the main mechanism [2].The use of agents that do not kill pathogenic bacteria but modify them to produce a phenotype that is susceptible to an antibiotic could be an alternative approach to the treatment of infectious diseases. Such agents could render the pathogen susceptible to a previously ineffective antibiotic, and because the modifying agent applies little or no direct selective pressure, this concept could slow down or prevent the emergence of resistant genotypes [3].

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A similar approach can be used to target modifying enzymes and efflux systems.A number of *in vitro* studies have shown plant extracts to significantly reduce the minimum inhibitory concentrations (MICs) of antibiotics against some resistant pathogenic bacteria [4], [5], [6], [7]. It is speculated that inhibition of drug efflux and alterations in membrane permeability could be responsible for the synergistic interactions between plant extracts and antibiotics [8]. The use of plant constituents in a bid to fight against the spread of antibiotic resistant pathogens is still an untapped resource [9].

Acanthospermum hispidum(DC), (family Compositae) is used in traditional medicine for the treatment of jaundice, malaria, stomachache, constipation, fever, [10] and viral infections [11]. The ethanolic extracts of the leaves and flowering tops have showed varying degrees of activity against a wide range of pathogenic bacteria [12]. We hereby report on the effect of this plant extract on the antibacterial activity of some antibiotics.

2. **Materials and methods**

2.1. **Plant material.**

The aerial parts of *Acanthospermum hispidum* were collected from the KNUST Campus, and identified in the Department of Pharmacognosy where a voucher specimen (FP/094/10) has been deposited. They were washed and sun dried for 5 days and then milled into coarse powder using a laboratory Mill Machine (Type 8, Christy & Norris, UK).

Three hundred and fifty grams of the powder was Soxhlet extracted using 70% ethanol and concentrated under reduced pressure using a Buchi Rotavapor R-114. The concentrate was evaporated to dryness at 40^{o}C in a hot air oven. The extract (38.7g; yield 11.0%) was stored in an airtight container at 4°C.

2.2. **Microorganisms used for the tests**

organisms used for the tests were: *Staphylococcus aureus* ATCC 25923 *Klebsiella pneumoniae* ATCC 31488, *Bacillus subtilis*, NCTC 10073, *Salmonella typhi* ATCC 19430, *Proteus vulgaris* NCTC 4635 *Pseudomonas aeruginosa* ATCC 27853. These were subcultures from of the stocks kept in the Pharmaceutical Microbiology Laboratory, KNUST, Kumasi.

2.3. **Antimicrobial activity determination**

The antimicrobial activity was determined using the Kirby-Bauer agar disc diffusion method [13].
25 ml Muller Hinton agar (Sigma-Aldrich, St Louis, MO, USA) plate was poured and allowed to set. 10 µl of the bacterial culture, diluted to 0.5 McFarland standards with saline, was spread over the surface using a spreader.
400 mg/ml solution of the extract was prepared in 50% methanol and serial dilutions were made to produce 300 mg/ml, 200 mg/ml and 100 mg/ml solutions.

Filter paper discs (6 mm) soaked with 30 µl of the various concentrations of the extracts as prepared above were placed at various marked positions. The test plate was allowed to stand for one hour and then incubated at 37^{o}C for 24 hours. The 50% methanol was also tested as a control.

The minimum inhibitory concentrations (MIC) of the extract against the various organisms were then calculated from semi-log plot of values of concentration and mean zones of inhibition.

3. **Antibiotic resistance modulation assay**

In the resistance modulation assay, the MICs of the antibiotics (amoxicillin and ciprofloxacin, Sigma) against the various organisms were determined by the agar disc diffusion method as above. The MICs of the antibiotics were re-determined by using a sub-inhibitory concentration of the extract (5mg/ml) as solvent for the preparation of the various concentrations of the antibiotics.

4. **Results**

The ethanolic extract of *A. hispidum* showed antimicrobial activity against all the test microorganisms (Table 1). *B. subtilis* was the most sensitive organism while *Ps. aeruginosa* was the least sensitive.

Table 1 Minimum Inhibitory Concentrations (MIC) of *A. hispidum* extract against organisms

Organisms	MIC (mg/ml)
S. aureus	44.7
B. subtilis	11.0
K. pneumonia	48.0
P. aeruginosa	52.5
P. vulgaris	39.0
S. typhi	39.0

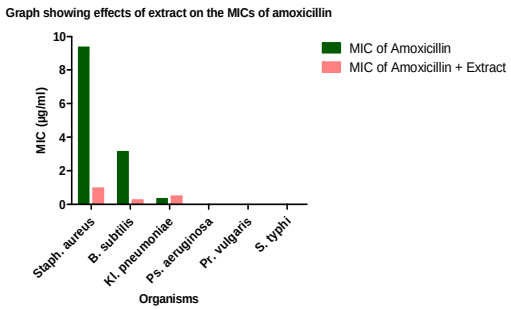

Figure-1

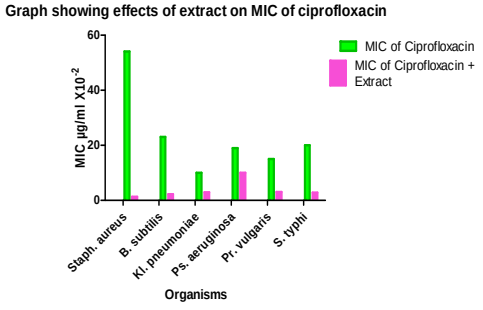

Figure-2

5. **Discussion**

Acanthospermum hispidum has been found to possess antimicrobial activity [12]. The crude ethanol extract of *A. hispidum* showed varying degrees of activities (table 1) against all the test organisms used. The plant extract was most active against *B subtilis* aqnd least active against *Ps aeruginosa.*

The extract demonstrated resistance modifying activity with both amoxicillin and ciprofloxacin.

Amoxicillin is a β-lactam antibiotic. Susceptibility to β-lactam antibiotics reflects the combined effects of binding to targets (penicillin-binding proteins [PBPs]), stability to β-lactamases, and, in Gram-negative bacteria, outer-membrane permeability. Similarly, resistance reflects a change in any of the three components [14].

Penicillin binding proteins (PBPs) are present in almost all bacteria, but they vary from species to species in number, size, amount, and affinity for β-lactam antibiotics, usually following taxonomic lines [14]. They are localized non-randomly on the outer face of the cytoplasmic membrane [15] and are anchored through short hydrophobic carboxy- or amino-terminal sequences; in Gram-negative bacteria, they are pseudoperiplasmic [16].
Amoxicillin combined with the plant extract produced a significant resistance modifying activity ($p<0.05$) against *Staph. aureus* (9 fold increase in activity) and *B. subtilis* (12 fold increase in activity), both Gram positive bacteria (Fig. 1). There was a slight reduction in the activity of amoxicillin against *Kl pneumoniae* (Gram negative bacteria). Amoxicillin showed no activity against *Ps. aeruginosa*, *Pr. vulgaris,* and *S. typhi* (Gram negative organisms) at the concentrations used both in the presence and absence of the extract.
Traditionally, the major mechanism of β-lactam resistance has involved β-lactamases, particularly plasmid-mediated β-lactamases. In Gram-negative bacteria, β-lactamases are periplasmic and act in combination with altered outer membrane permeability. In Gram-positive bacteria, they are exocellular, although they are probably associated with the cell wall through electrostatic interactions [17].

The major enzymatic activities associated with PBPs are peptidoglycan transpeptidase, which is believed to be essential, and DD-carboxypeptidase, which is believed to be dispensable. In a given organism, there are two to four essential PBPs and, thus, potentially multiple β-lactam targets. Altered PBPs associated with β-lactam resistance are more commonly found in Gram-positive than in Gram-negative bacteria [14].

This suggests that the resistance modifying activity exhibited by the extract on amoxicillin may be due to constituents that act on the cell wall, which probably, inhibit β-lactamase activity, makes the cell wall more permeable or enhances the interaction between amoxicillin and target sites.

The primary target of all the fluoroquinolones including ciprofloxacin is DNA gyrase (topoisomerase II), [18].

Two mechanisms of quinolone resistance have been described; alterations in the targets of quinolone and decreased accumulation due to impermeability of the membrane and/or over expression of efflux pump systems. Mobile elements have also been described carrying the *gnr* gene which confers resistance to quinolones [19].

A combination involving Ciprofloxacin with the sub-inhibitory concentrations (5mg/ml) of *A. hispidum* extract produced MICs that are significantly lower than the standard drugs alone ($p<0.05$) against all the bacteria used (Fig. 2). There was a high increase in activity (38 fold decrease in MIC) against *Staph. aureus* and *B. subtilis* (10 fold). There were increases in activity against *S. typhi*, *Pr. vulgaris*, and *Kl. pneumoniae* (7 fold, 5 fold, 3 fold respectively).

There was only a marginal decrease in activity (< 2 fold) against *Ps. aeruginosa.* As in the case of amoxicillin the resistance modifying activity is more pronounced with Gram positive organisms than the Gram negatives.

The results of the study pre-suppose that the effects of the extracts are on the cell wall peptidoglycan permeability. Increasing permeability of the cell wall enhanced the activity of both drugs against Gram positive organisms (which contain 50-90% peptidiglycan) because of better interaction between antibiotic and organism. For Gram negative organisms which have much less peptidoglycan (5-10%) the effects were minimal [20].

These results suggest that, there is possibly some phytochemical components in the ethanol extract of *A. hispidum* which affected one or more of the resistance mechanisms of these organisms probably the efflux mechanisms or increased the binding of the antibiotic to target cells and as such, increased the efficacy of the drugs. This is similar to the observations made by [5].

6. **Conclusion**

Sub-inhibitory concentrations of *A. hispidum* (5mg/ml) enhanced the activity of amoxicillin against *Staph. aureus* and *B. subtilis* but reduced slightly the activity against *Kl. pneumoniae*. Combining ciprofloxacin with the sub-inhibitory concentrations (5mg/ml) of *A. hispidum* extract modulated the resistance of all the organisms to ciprofloxacin. The resistance modulatory activity of the extracts on amoxicillin and ciprofloxacin is more pronounced with Gram positive organisms than Gram negative organisms.

The findings show that it is possible to find a phytoconstituent in *A. hispidum*, which in combination with ciprofloxacin and amoxicillin will produce a regimen that is capable of managing infections due to organisms that are resistant especially to ciprofloxacin.

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Formulation and evaluation of Venlafaxine HCl microspheres

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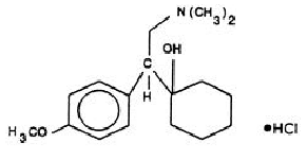
Abstract:

Venlafaxine HCl is the freely water soluble drug with antidepressant activity. It is available in a dose of 25mg-45mg. Being highly soluble drug there is possibility of burst effect which causes sudden peak levels of drug in blood. It is having many side effects and with a half life around 5 hours. To reduce the adverse effects due to burst effect and to have the sustain action of the drug we have prepared the ethyl cellulose microspheres of the drug. The microspheres were prepared by the emulsification and solvent evaporation method. The prepared microspheres were evaluated mainly for the sustain release of the drug and then the anti depressant activity in albino mice apart from the other tests like % drug encapsulation, particle size and drug polymer compatibility by the FTIR studies. The method had resulted in good encapsulation efficiency and micron sized ethyl cellulose spheres. The drug release was found to be sustained for 16 hours and was found to follow the Peppas kinetics.

***Key words*:** Venlafaxine HCl, anti depressant activity, ethyl cellulose microspheres, sustain release dosage forms.

1. **Introduction**

Venlafaxine HCl , commercially known as “Effexor” is a representative of a new class of antidepressants. It is a bicyclic phenylethyl amine and chemically unrelated to tricyclic, tetracyclic or other available antidepressant agents and designated as (R/S)-1-[2-dimethyl amino)-1-(4-methoxy phenyl) ethyl] cyclohexanol hydrochloride. This medication is used to treat anxiety.



venlafaxine hydrochloride

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Venlafaxine HCl is a water soluble drug with a solubility of 572mg/mL[1]. By preparing the sustain release dosage form, the initial burst effect of highly water soluble drugs like Venlafaxine HCl can be prevented.

It acts by inhibiting selectively the uptake of serotonin and nor-adrenaline but shows no affinity for neurotransmitter receptors[2]. It has many adverse effects like nausea, asthenia, dizziness, insomnia, somnolence, headache, dry mouth, sweating, hypotension, nervousness and abnormal ejaculation. The half life of Venlafaxine HCl and its active metabolite O-desmethyl venlafaxine are 5hr and 11hr respectively[3]. A Venlafaxine HCl overdose may be more serious than an overdose with selective serotonin reuptake inhibitors[4].

Antidepressants have characteristics that make the drugs suitable for controlled-release formulations, which might have advantages over immediate-release formulations. For example, controlled-release formulations are associated with lower peak plasma drug concentrations and less fluctuation between peak and trough plasma drug concentrations. In addition to having more stable pharmacokinetic profiles, some controlled-release formulations are associated with lower incidences of nausea than are immediate-release formulations of the same medications. Therefore, some patients who experience intolerable nausea with an immediate-release formulation despite seeing improvement in their depressive symptoms might benefit from taking a controlled-release formulation of the same antidepressant or switching to another of the newer antidepressants. The serious morbidity associated with untreated or inadequately treated depression implies that major benefits may occur in the quality of life for patients who can be salvaged from discontinuing therapy with the use of the most tolerable drug formulations[5].

The main object of our study is to prepare a sustained release dosage form and thus to reduce the complications of Venlafaxine HCl overdose.

2. **Materials and Methods:**

Venlafaxine HCl was a gift sample from Dr. Reddy's, ethyl cellulose, low viscosity grade (250cps in 2% solution at 25ºC) liquid paraffin and span-80 were purchased from SD Fine chemicals (Mumbai), all other chemicals & solvents were of analytical grade.

2.1. **Preparation of the microspheres:**

Two formulae were prepared by taking drug: polymer ratio of 1:1 and 1:2. The microspheres were prepared by this method and named as F1 and F2 respectively. **I**n this method 50 mL of acetone was taken, to this required quantity of ethyl cellulose was added and dissolved. To this drug was added. This dispersed phase was added to continuous phase (100mL) consisting of liquid paraffin containing 0.5% (w/v) span-80 to form water in oil (w/o) emulsion. Stirring was continued at 2000 rpm using a magnetic stirrer for 2 hrs to obtain microspheres.

The microspheres were separated by filtration and washed first with petroleum ether and then with distilled water to remove the adhered liquid paraffin. The microspheres were then finally dried at room temperature.

2.2. **Determination of micromeritic properties.**

The mean particle size of the ethyl cellulose micro spheres was determined by the sieving method[6].

2.2.1. *Entrapment efficiency of the drug*

The micro spheres equivalent to 10mg of Venlafaxine HCl were weighed and dispersed in PBS of pH 7.4. The resulting mixture was agitated on mechanical shaker for 24 hours. The solution was then filtered and drug content was estimated by UV spectrophotometry.

2.3. *FTIR study*

IR spectra of the Venlafaxine HCl, pure ethylcellulose and the drug with ethylcellulose were obtained and were compared for the compatibility.

2.4. *In vitro drug release*

The in vitro release of the drug from microspheres was studied in Phosphate buffer of pH 7.4 (900mL) by using USP basket type dissolution rate test apparatus (ELECTROLAB-TDT 08L) at 100rpm. Microspheres equivalent to 100mg of Venlafaxine HCl were used in each test. Samples were withdrawn at different time intervals and assayed by single beam UV Spectrophotometer (ELICO SL 159) at 229nm.

2.5. *Model fitting for drug release*

The suitability of several equation that are reported in the literature to identify the mechanisms for the release of drug was tested with respect to the release data up to the first 50% drug release. The data were evaluated according to the following equations:

Zero order model [7]

$M_t = M_0 + K_0 t$

Higuchi model[8, 9]

$M_t = M_0 + K_H t^{0.5}$

Korsmeyer-Peppas model[10, 11]

$M_t / M_\alpha = K_K t^n$

Where Mt is the amount of drug dissolved in time t. M_0 is the initial amount of the drug. K_0 is the Zero order release constant, K_H is the Higuchi rate constant, K_K is a Peppas release constant and n is the release exponent that characterizes the mechanism of drug release.

3. **Anti depressant activity:**

Animals:

Albino mice of either sex weighing between 20-25g bred in central animal house of Nalanda College of Pharmacy were used. The animals were housed under standard laboratory conditions and maintained on natural light and dark cycle and had free access to food and water.Animals were acclimatized to laboratory conditions before the experiment. Each animal was used only once. The experiments were conducted according to the CPCSEA guidelines.

Phenobarbitone induced hypnosis:

It is one of the animal models to test the sleep promoting or sedative properties of the drug. Phenobarbitone Sodium (45mg/kg I.P.) was used to induce sleep. The sleep time (onset of sleep) and duration of sleep were recorded after giving the venlafaxine of 10mg/Kg equivalent microspheres (F1) orally[12].

4. **Results and Discussion**

The prepared micro spheres were almost spherical in shape and these are having the average particle sizes of 65.60 ± 0.255 and 126.39 ± 0.502 for the formulations F1 and F2 respectively where n=3. This indicates that the increase in the polymer concentration has increased the average particle size of the micro spheres and this may be due to the reason that the viscosity of the solution was high with increase in the polymer concentration. The micro photograph picture of the Venlafaxine micro sphere was shown in the figure 1. The drug entrapment efficiency was found to be 77.87%± 0.325 and 81.56%±1.254 for F1 and F2 respectively. The entrapment efficiency also got increased with increase with the ethyl cellulose concentration.

IR spectra of the Venlafaxine HCl, polymer and the drug along with polymer have revealed that there is no shifting of the peaks indicating the compatibility of the polymer with the drug Venlafaxine HCl. The FTIR data was given in Figure 4.

The release profile of Venlafaxine HCl from micro spheres exhibited more sustained release with increased polymer concentration and release rate was found in the order of F1‹ F2. This indicates decreased drug release rate is due to increased thickness of the polymer[13]. Statistical data was presented in table no. 1 for F1.

In order to determine the mechanism of drug release from the micro spheres the dissolution data was put in to various models and among them the formulation F1 was best fit in to Korsmeyer-Peppas equation indicating that the drug release is following the non-fickian diffusion. The ‘n’ value for the Korsmeyer- Peppas equation was 0.7550. Details were given in table no.2. In the antidepressant activity evaluation it was found that the sleeping time with F1 was decreased by 45 minutes and the onset of sleep was late by 3 minutes.

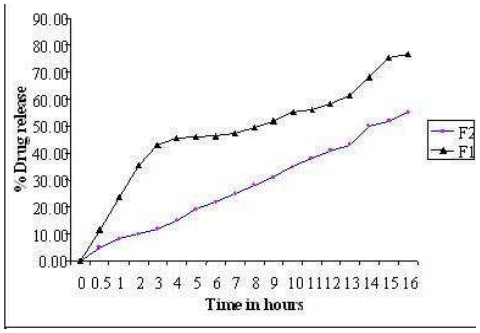


Fig. 2 Comparison of % drug release from formulation F1 and F2 in PBS 7.4

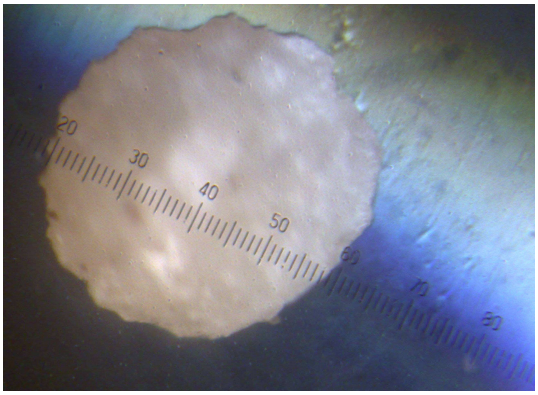


Fig. 1 Microphotograph Venlafaxine ethyl cellulose microsphere.

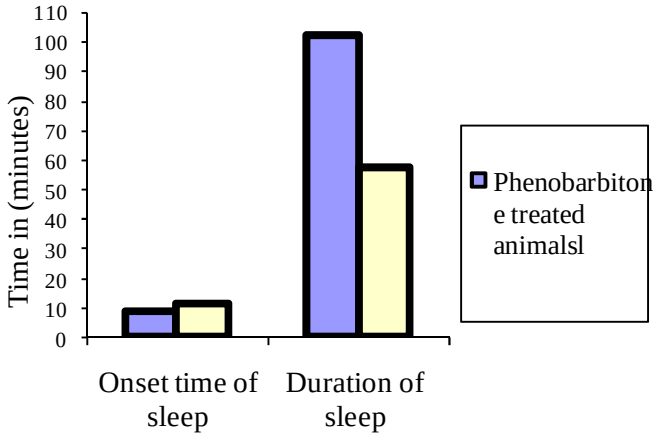


Fig. 3 Onset time and duration of sleep in mice injected with Phenobarbitone (I P) followed by the micro spheres of the drug Vs Distilled water as control.

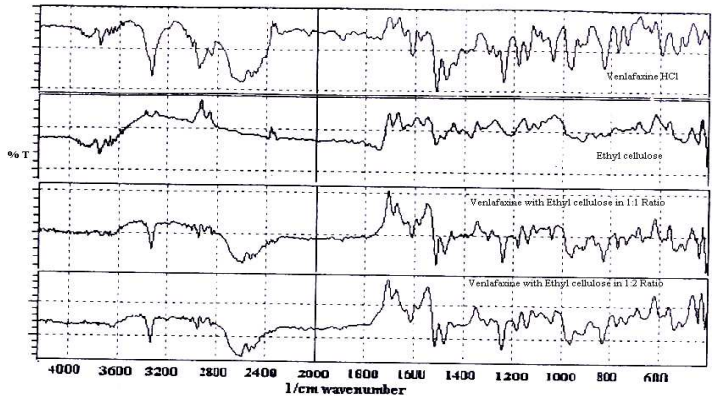
Table 1. Statistical dissolution data for formulation 1

Sr.No.	Time (Hrs)	Avg. %R	SD	SE-mean	RSD
1	0	0.000	0.00	0.00	0.00
2	0.5	11.612	0.27	0.19	2.36
3	1	23.709	0.11	0.08	0.46
4	1.5	35.325	0.65	0.46	1.84
5	2	42.762	0.65	0.46	1.52
6	2.5	45.671	0.16	0.11	0.35
7	3	45.940	0.27	0.19	0.58
8	3.5	46.203	0.16	0.11	0.34
9	4	47.131	0.58	0.41	1.23
10	4.5	49.233	0.37	0.26	0.75
11	5	51.861	0.31	0.22	0.60
12	6	55.334	0.16	0.11	0.28
13	7	55.957	1.19	0.84	2.12
14	8	58.089	0.15	0.11	0.26
15	10	61.346	0.46	0.32	0.75
16	12	68.032	0.61	0.43	0.89
17	14	75.274	0.05	0.04	0.07
18	16	76.657	1.65	1.17	2.15

Table 2. Model fitting data for both the formulations

S.No	*Zero- order Equation*		*Higuchi Equation*		*Korsmeyer-Peppas Equation*			First –order Equation	
	K_0	*R*	K_H	*R*	K_K	*n*	*R*	K_1	R
F1.	7.440	0.8481	23.960	0.9175	22.6414	0.7550	0.9687	-0.0299	-0.9311
F2.	2.1980	0.9118	10.2974	0.8954	10.6074	0.4797	0.7682	-0.1865	-0.9410

Fig 4: FTIR spectra of Venlafaxine HCl with Ethyl cellulose.



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In-Vitro Antimicrobial Effects of Some Selected Plants against Bovine Mastitis Pathogens

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Abstract

Bovine mastitis continues to be the most costly disease to the dairy farmers. It dominates in Tamilnadu as one of the most prevalent diseases in dairy cattle among the dairy farms. Mastitis treatment with antibiotics leads to the development of antibiotic resistant strains and consumer health problem. The present study is an *in vitro* antibacterial activity of three medicinal plants against bovine udder isolated bacterial pathogens. Aqueous and methanol extracts of three plants were investigated by agar disc diffusion method. Methanol extracts of *Asteracantha longifolia* and *Dactyloctenium indicum* showed significant activity against *Staphylococcus aureus* (25mm) and *E.coli* (22mm) respectively.

***Key words*:** Bovine mastitis pathogens, Methanol, Disc diffusion method, *Asteracantha longifolia*, *Dactyloctenium indicum* and *Trichodesma indicum*

1. Introduction

Mastitis continues to be among the costliest diseases to the dairy industry, and annual economic losses attributed to this disease in the United States are estimated to approach $2 billion. Among cattle diseases, bovine mastitis is a serious problem which affects the basic income of the farmers depleting their dairy sources. Worldwide, mastitis is associated with economic losses of $35 billion annually. It adversely affects milk production whereby losses due to subclinical mastitis are more severe than those due to clinical cases. Controlling subclinical mastitis can reduce the losses in milk production substantially. Decreased milk production and quality, as well as veterinary expenses, all contributes to these economic losses [1].

Clinical and subclinical cases of mastitis are routinely treated with antimicrobials both intramammarily and parenterally. The use of antimicrobials over long periods has triggered the development of multidrug resistant strains, which has resulted in the use of increasing doses of antimicrobials, causing the danger of increasing amounts of drug residues in milk, a potential biohazard [2].

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Medicinal plants have been used for ages in developing countries as alternative treatment to health problems. India has a diverse flora and a rich tradition in the use of medicinal plants for antimicrobial applications. In India specifically in Tamil Nadu ethnoveterinary practices are very common in villages. Most of the approaches of the farmers are based on empiric knowledge with significant results in cattle. A short survey prior to this study was undertaken among known farmers about their interest in ethnobotany and treatment of their cattle sources. Most of them expressed a desire to learn more about the proper use and application of ethnoveterinary practices as these were economically, socially and culturally more acceptable for marginalized communities.

The present study was undertaken to investigate the effects of aqueous and methanolic extracts of *Asteracantha longifolia*, *Dactyloctenium indicum* and *Trichodesma indicum*. To our knowledge, no reports or studies exist relating to *in vitro* application of *A. longifolia*, *D. indicum* and *T. indicum* extracts in bovine mastitis studies. This is the first report on *A. longifolia*, *D. indicum* and *T. indicum* antibacterial action against bovine mastitis isolated contagious pathogens.

2. **Materials and Methods**

2.1. *Plant collection*

Fresh plant parts of *Asteracantha longifolia*, *Dactyloctenium indicum* and *Trichodesma indicum* were collected randomly from the gardens and villages of Coimbatore district, Tamilnadu, India. The taxonomic identities of plants were confirmed by Dr.V.Sampath Kumar, Scientist, Botanical Survey of India (Southern Circle), Coimbatore, Tamilnadu, India and the voucher specimen of the plant was preserved in RVS College Microbiology Laboratory. The collected plants were washed with running tap water, air dried, homogenized to a fine powder and stored in air-tight bottles at 4^{o}C.

2.2**.** *Preparation of Crude Extracts*

Solvent extraction

100 grams of dried plant material was extracted with 200 ml of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume [3]. It was stored at 4^{o}C in airtight bottles for further studies.

Aqueous extraction

100 grams of dried plant material was extracted in distilled water for 6 h at slow heat. Every 2 h it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected. This procedure was repeated twice and after 6 h the supernatant was concentrated to make the final volume one-fifth of the original volume [3].

2.3. *Bacterial strains*

Bacterial strains used in this study were the isolated pathogens isolated from clinical cases of bovine mastitis such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Streptococcus agalactiae.* All the strains were confirmed by cultural and biochemical characteristics [4] and maintained in slants for further use.

2.4. *Antibacterial activity*

The antibacterial assay of aqueous and methanolic extracts was performed by Bauer *et al.* [5]. The Mueller Hinton Agar media, along with the inoculum (10^6 cfu/ml) was poured into the petridishes. For the agar disc diffusion method, the disc (0.7 cm) (Hi-Media) was saturated with 100 mg/ml of the test compound, allowed to dry and then placed on the upper layer of the seeded agar plate. The plates were incubated overnight at 37°C. Antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm) surrounding bacterial growth. For each bacterial strain, controls were included that comprised pure solvents instead of the extract [6].

3. **Results and Discussion**

The traditional ethno-veterinary medicinal practices are being followed by the rural folk through which a number of veterinary diseases are managed in the developing countries. The use of antibiotics and other chemical products are banned for animal healthcare in a number of countries because of human healthcare. The World Health Organization (WHO) states that 74% of the medicines derived from plant resources have a modern indication that correlates with their traditional, cultural (and sometimes ancient) uses [6].

Results obtained in the present study revealed that the tested three plant extracts posses' potential antibacterial activity against *S.aureus, E.coli,* S. *agalactiae* and *K.pneumoniae* (Table 1). Each plant extract of the three plant species were tested at two different concentrations (100 & 200 mg/ml) to see their inhibitory effects against bovine mastitis isolated pathogens. Of the three candidate plants in this study, *A.longifolia* showed significant antibacterial activity against all the tested bacteria and the remaining plants showed moderate activity after alcoholic extraction. None of the extracts showed activity against *K.pneumoniae*.

The most pronounced activity with inhibition zones of more than 14.0 mm was shown by methanol extract (inhibition zone 25 mm against *S.aureus* at concentration 200mg /ml) and aqueous extract (inhibition zone 16 mm against *E.coli* at concentration 200mg/ml) of *A. longifolia*. The methanol extract of *D.indicum also* showed significant antimicrobial activity against *Staphylococcus aureus* and *E.coli* with inhibition zones 22 and 20 mm respectively at concentration 200 mg /ml while the aqueous extract showed inhibition against *E.coli* with 18mm inhibition zones at concentration 200 mg/ml. When the concentration of the extracts were decreased from 200-100 mg/ml slight decrease in inhibition zones were observed.

Wynn [7] describes today's traditional medicine, as undoubtedly the oldest form of medicine and probably had evolved simultaneously with the evolution of human beings. EVM has been a mainstay of developing countries that lack access to conventional medicines for veterinary health care, often the only unaffordable means to poor farmers. The Ethno veterinary medicine (EVM) practices could be an effective approach for tackling problems like mastitis, bovine viral diarrhea and many deficiency disorders. With the traditional knowledge in the background potential plants can be prospected to reach the active fraction or molecule(s), which can be further formulated. Besides, the dried plant material itself could be utilized, by premixing it with the fodder of cattle feed thereby utilizing the pure molecule indirectly as a marker to maintain the product quality control. Further studies may be necessary to elucidate the specific phytoactive compounds in the leaf extract of the plant *A. longifolia* and *D. indicum*

Acknowledgement

The first, second and third authors are grateful to University Grant Commission (UGC) for the financial support given to the present study under the Major Research Project programme entitled ”A Study of Ethno-veterinary Medicinal Plants and *in-vitro* antimicrobial activities against Bovine Mastitis isolated bacterial pathogens” [Sanction No. F. No. 35-121 / 2008 (SR) dt.20 March 2009]. The authors are thankful to the management of RVS Educational Trust for their encouragement and support.

Table 1: Antibacterial activity of ethno veterinary medicinal plants

Medicinal Plants	**Extracts**	**Conc. (mg/ml)**	**Zone of Inhibition (mm)**			
			S.aureus	*E.coli*	*S. agalactiae*	*K. pneumoniae*
A. longifolia	Methanol	100	18.7 ± 0.3	14.1 ± 0.1	-	-
		200	24.2* ± 0.52	16.7 ± 0.25	9.0 ± 0.1	-
	Water	100	11.6 ± 0.55	12.0 ± 0.05	-	-
		200	13.7 ± 0.25	15.7 ± 0.26	-	-
D. indicum	Methanol	100	19.8 ± 0.28	16.8 ± 0.15	10.0 ± 0.05	-
		200	21.7* ± 0.25	20.0* ± 0.05	10.7 ± 0.25	-
	Water	100	10.9 ± 0.1	13.8 ± 0.32	-	-
		200	14.5 ± 0.5	17.8 ± 0.20	9.7 ± 0.251	-
T.indicum	Methanol	100	-	10.7 ± 0.26	-	-
		200	14.8 ± 0.28	14.9 ± 0.11	15.8 ± 0.152	-
	Water	100	-	-	-	-
		200	-	10.8 ± 0.28	-	-
Standard (Ciprofloxacin)		10µg	29±0.1*	30±0.15*	22±0.1*	20±0.15*

n=3, student ‘t’ test, * p value < 0.01, ** p value < 0.01

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A study on the nutraceuticals from the genus *Rumex*

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Abstract:

The genus *Rumex* is found to be distributed worldwide. This genus includes more than 250 species. Most of the species under this genus contain phytoconstituents like, flavonoids, anthraquinones and triterpenoids. Many species are herbs but shrubs also included. Roots are usaually taproot but a few are having rhizomes. Very few of them are explored scientifically *(Rumex patentia, Rumex japonicas, Rumex hymenosepalus, Rumex crispus, Rumex dentatus)* .80% methanolic extract of rhizome*Rumex abyssinicus* reported to have diuretic and analgesic activity.The triterpenoids which are isolated from the *Rumex japonicus* shows Rat lens Aldose reductase inhibitory activity.Leucodelphinidin and Leucopelargonidin isolated from the *Rumex hymenosepalus* identified as a antitumourous substances.Neopodin which is isolated from the ethanolic extract of *Rumex japonicus*exhibited the inhibitory activity of osteoclast.This paper presents the morphological features,chemical constituents and uses of the different reported species and folklore uses of *Rumex* as these can be explored as potential Nutraceuticals.

Key words: Rumex species, anthraquinones, triterpenoids, flavonoids, diuretic, Nutraceuticals

1. Introduction:

About 200 species (Table-2) widely distributed in North and south temperate zones; 27 species (one endemic) in china[1], twelve species of *Rumex* occur in Texas[2.] *Rumex acetosa, R. acetosella, R. alpestris (R. arifolius), R. auriculatus, R. aviculare, R. hastatus, R. lunaria, R. longifolius, R. montanus, R. patienta, R. polyanthemus, R. repens, R. scutatus, R. thyrsifolius, R. tuberosus, R. vesicarius*. grows in Africa: Libya, Morocco, and South Africa . Asia-Temperate: Azerbaijan, Republic of Georgia, Israel, Kazakhstan, Kirghizistan, Stavropol. Asia-Tropical: India. Australasia: New Zealand. Europe: Austria, Cyprus, Czechoslovakia, Denmark, Eire, Estonia, Faeroes, Finland, France, Germany, Hungary, Italy, Latvia, Norway, Poland, Romania, Russia (Novgorod, St. Petersburg, Smolensk, Yaroslavl), Serbia, Sweden, Switzerland, Ukraine, UK (England, Scotland), Yugoslavia[3].

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But in India many species of *Rumex* have been reported in chattisgarh, among all the species *Rumex vesicarius* is most popular[4].This paper is aimed to report the Morphology, Chemical constituents, Reported and traditional uses (Table-1) of some species of Rumex.

2. **General Morphology** [1]

Herbs are perennial or less commonly annual, rarely shrubs, rarely dioecious. Roots are usually stout (taproots), or sometimes plantsrhizomatous. Stems erect, ascending to prostrate, branched, not hollow or sulcate. Leaves simple, often dimorphic, fugaciousor persisting, basal and cauline, alternate, margin entire or undulate; ocrea tubular, membranous, margin entire. Inflorescence is usuallyterminal, sometimes terminal and axillary, racemose or paniculate. Pedicel articulate (the functional pedicel consists of the truepedicel and, below the joint, the narrowed united basal parts of the outer tepals (pseudopedicel)). Flowers are bisexual or unisexual (unisexual in dioecious, and rarely in polygamo-monoecious plants). Perianth persistent, tepals 6, becoming enlarged and oftenhardened in fruit; valve (fruiting inner tepal) margin entire, erose, denticulate, or variously dentate, midvein often transformed intotubercles (tuberculate callosities). Stamens 6. Styles 3, elongate; stigmas penicillate. Achenes trigonous, elliptic to ovate.

Morphological Description of Some Species of*Rumex*[1]

Rumex acetosella[1]*:* Herbs perennial, dioecious. Rhizomes horizontal, ligneous.Stems usually numerous from rhizome, erect or ascending, 15-35(-45) cm tall, slender, finely grooved, branched above middle.Basal leaves hastate, rarely without basal leaves, 2-4 cm ×3-6(-10) mm, glabrous, central lobe ovate-lanceolate, lanceolate,or linear, basal lobes spreading or curved, sometimes multifid, margin above basal lobes entire, apex acute or obtuse;cauline leaves smaller upward. Petiole short or in upper cauline leaves nearly absent; ocrea fugacious, white, membranous.Inflorescence terminal, paniculate. Flowers unisexual. Pedicel 2-2.5 mm, articulate near base of tepals. Male flower: outer tepals small; inner tepals elliptic, ca. 1.5 mm. Female flower: outer tepals lanceolate, ca. 1 mm, not reflexed in fruit; inner tepals slightly enlarged in fruit; valves ovate, 1-1.6 mm, without tubercles, net veined, base rounded to broadly cuneate, margin entire, apex acute. Achenes brown, shiny, broadly ovoid,trigonous, 1-1.5 mm

Rumex hastatus[1]*:* Shrubs 50-90 cm tall. Branches purple-brown, finely grooved; branchlets green, glabrous. Leaves solitary or fascicled; petiole 1.5-3.5 cm; leaf blade hastate, 1.5-3 cm × 1.5-2 mm, subleathery, central lobe linear or narrowly triangular, apex acute, basal lobes curved; ocrea fugacious, membranous.Inflorescence terminal, paniculate, lax. Pedicel slender, articulate below middle. Flowers polygamous. Male flowers: tepals nearly uniform. Female flowers: outer tepals elliptic, reflexed in fruit; inner tepals enlarged in fruit; valves pinkish, orbicular or reniform, membranous, nearly pellucid, with small tubercle at base, base deeply cordate, margin nearly entire, and apex obtuse or retuse. Achenes brown, shiny, ovoid, trigonous, ca. 2 mm.

Rumex acetosa[1]*:* Herbs perennial, dioecious, with a short and relatively thin horizontal or slightly oblique rootstock, usually not reaching deep into substrate and with rather crowded secondary roots. Stems erect, 40-100 cm tall, grooved, glabrous, usually simple.

Basal leaves ovate-lanceolate to lanceolate, base sagittate, 3-12 × 2-4 cm, margin entire, apex acute, basal lobes acute at apices; cauline leaves small; petiole short or nearly absent; ocrea fugacious, white, membranous. Inflorescence terminal, paniculate, lax; branches reddish green, slender, simple or with a few secondary branches. Flowers unisexual. Pedicel slender, articulate at middle. Male flowers: outer tepals erect, small; inner tepals elliptic, ca. 3 mm. Female flowers: outer tepals elliptic, reflexed in fruit; inner tepals enlarged in fruit; valves nearly orbicular (to broadly ovate), 3.5-4 mm in diam., with small recurved tubercles at base of valves, net veined, base cordate,margin entire, apex obtuse. Achenes blackish brown, shiny,Ellipsoid, trigonous, ca. 2 mm.

Rumex thyrsiflorus[1]*:*Herbs perennial, dioecious. Taproots large, thick, with remote secondary roots. Stems erect, 40-120 cm tall, glabrous, grooved. Basal leaves oblong-lanceolate to lanceolate, base sagittate, 4-13 × 1.5-4 cm, both surfaces glabrous or veins minutely papillate, margin entire, apex acute, basal lobes acute at apex; cauline leaves small; petiole short or nearly absent; ocrea fugacious, white, membranous. Inflorescence terminal, paniculate, dense, much branched. Flowers unisexual. Pedicel slender, articulate below middle. Male flowers: outer tepals erect, small;inner tepals elliptic, ca. 2 mm. Female flowers: outer tepals reflexed in fruit; inner tepals enlarged in fruit; valves orbicular to broadly ovate, 3-4 mm in diam., with small recurved tubercles at base of valves, base truncate to cordate, margin nearly entire, apex obtuse. Achenes brown, shiny, ellipsoid,trigonous, ca. 2 mm.

Rumex longifolius[1]*:*Herbs perennial. Stems erect, 60-120 cm tall, robust, glabrous, grooved, branched above middle. Basal leaves: petiole 5-15 cm; leaf blade oblong-lanceolate or broadly lanceolate, 20-35 × 5-10 cm, abaxially minutely papillate along veins, adaxially glabrous, base cuneate or rounded, margin slightly undulate to weakly crisped, apex acute or subacute; cauline leaves shortly petiolate, lanceolate, small, base narrowly cuneate, apex acute; ocrea fugacious, white, membranous. Inflorescence paniculate. Flowers bisexual. Pedicels slender, articulate below middle; articulation distinctly swollen in fruit. Inner tepals enlarged in fruit; valves broadly orbicular-reniformto orbicular-cordate, 5-6 × 6-7 mm, all without tubercles, sometimes 1 valve with small indistinct tubercle, net veined, base cordate, margin entire, apex obtuse. Achenes brown, shiny, narrowly ovoid, trigonous, 2-3.5 mm.

Rumex angulatus[1] *:* Herbs perennial. Stems erect, purple-red, 40-60 cm tall, glabrous, grooved. Basal leaves: petiole 3-5 cm; leaf blade oblong-lanceolate, 15-20 × 3-5 cm, both surfaces glabrous, base cuneate, apex acute; cauline leaves shortly petiolate, lanceolate; ocrea fugacious, membranous. Inflorescence terminal, paniculate, dense; rachis slightly zigzagged. Flowers bisexual. Pedicel filiform, slender, articulate below middle. Inner tepals enlarged in fruit; valves orbicular-cordate, ca. 5 × 4 mm, all without tubercles, net veined, base cordate, margin nearly entire or irregularly minutely crenate, and apex obtuse. Achenes yellow-brown,shiny, ovoid, trigonous, ca. 3 mm.

Rumex pseudonatronatus[1]: Herbs perennial. Roots vertical, large, 1.2 cm in diameter. Stems erect, 80.120 cm tall, simple or branched above, grooved, glabrous. Basal leaves lanceolate or narrowly lanceolate, sometimes lanceolate-linear, 15.30 × 1.5.4 cm, abaxially minutely papillate along veins, adaxially glabrous, base cuneate to narrowly cuneate, margin crisped or undulate, apex acute; cauline leaves shortly petiolate, narrowly lanceolate, small; ocrea fugacious, white,

thinly membranous. Inflorescence paniculate, dense in distal part, sometimes interupted at base,20.40 cm, narrow. Flowers bisexual. Pedicels slender, articulate below middle, articulation swollen in fruit. Inner tepals enlarged in fruit; valves nearly orbicular or orbicular-cordate,3.5.4.5 mm, all without tubercles, sometimes 1 valve with 1 indistinct tubercle less than 1.1.3 mm, conspicuously net veined, base slightly cordate, margin entire or weakly erose, apex obtuse. Achenes brown, shiny, narrowly ovoid, trigonous,2-2.5 mm.

Rumex aquaticus[1]*:* Herbs perennial. Stems erect, 30-120 cm tall, usually branched above (in inflorescence), glabrous, grooved. Basal leaves: petiole 9-28 cm, glabrous or minutely papillate; leaf blade oblong-ovate to ovate-lanceolate, 10-30 × 4-13 cm, both surfaces glabrous or abaxially minutely papillate along veins,base cordate to nearly truncate, margin undulate, apex acute to nearly obtuse; cauline leaves shortly petiolate, oblong or broadly lanceolate, small; ocrea fugacious, membranous. Inflorescence terminal, paniculate, narrow; branches suberect. Flowersbisexual. Pedicel filiform, articulation indistinct, not swollen in fruit. Inner tepals enlarged in fruit; valves ovate, 5-8 × 4-6 mm, all without tubercles, base subtruncate, margin nearly entire, and apex acute. Achenes brown, shiny, ellipsoid, trigonous, 3-4.5mm, base narrow, apex acute

Rumex popovii[1]: Herbs perennial. Roots large, 0.8-1.5 cm in diameter. Stems erect, reddish, 60-100 cm tall, usually branched above, glabrous, grooved. Basal leaves: petiole 7-13 cm, stout; leaf blade oblong-ovate or narrowly ovate, 15-20 × 4-6 cm, both surfaces glabrous, base cordate, margin slightly undulate, apex acute; cauline leaves lanceolate; ocrea fugacious, membranous. Inflorescence paniculate; branches spreading. Flowers bisexual. Pedicel filiform, articulate below middle, articulation indistinct. Inner tepals enlarged in fruit; valves pinkish, nearly orbicular ororbicular-ovate, 4-5 mm in diam., all without tubercles, conspicuously net veined, base deeply cordate, margin inconspicuously denticulate. Achenes brown, shiny, ellipsoid, trigonous, ca. 2 mm.

Rumex yungningensis[1]: Herbs perennial. Stems erect, 70-120 cm tall, branched, glabrous, grooved. Basal leaves elliptic, 7-15 × 3-5 cm, abaxially minutely papillate along veins, adaxially glabrous, base cuneate, margin entire, apex acute; cauline leaves small; petiole short or nearly absent; ocrea fugacious, brown, thinly membranous. Inflorescence terminal, paniculate; rachis erect. Flowers bisexual. Pedicel filiform, 6-8 mm, articulates at base. Inner tepals enlarged in fruit; valves triangular-cordate, ca. 5 × 4 mm, all without tubercles, net veined, base deeply cordate, marginnearly entire, and apex obtuse. Achenes brown, shiny, narrowlyovoid, ca. 2.5 mm, apex acute

Rumex gmelinii[1]*:* Herbs perennial. Stems 40-100 cm tall, robust, glabrous, grooved. Basal leaves: petiole to 30 cm; leaf blade broadly triangular- ovate, 8-25 × 5-20 cm, abaxially densely papillate along veins, adaxially glabrous, base deeply cordate, margin entire or slightly undulate, apex obtuse; cauline leaves shortly petiolate, oblong-ovate, small, base cordate, apex obtuse; ocrea fugacious, membranous. Inflorescence paniculate. Flowers bisexual. Pedicels slender, articulate at base. Outer tepals ca. 2 mm; inner tepals enlarged in fruit; valves elliptic, 5-6 mm, allwith tubercles, net veined, base rounded, apex obtuse. Achenesdark brown, shiny, ovoid, trigonous, 2.5-3 mm.

Rumex patientia[1]*:* Herbs perennial. Roots vertical, large, to 3 cm in diameter. Stems erect, 80-150(-200) cm tall, robust, branched above, grooved. Basal leaves: petiole 5-15 cm, stout; leaf blade oblong or oblong-lanceolate, 15-30 × 5-10 cm, base rounded, broadly cuneate, or subcordate, margin undulate, apex acute to subacute; cauline leaves shortly petiolate or nearly sessile, lanceolate, small; ocrea fugacious, 2-4 cm, membranous. Inflorescence paniculate, large. Flowers bisexual. Pedicel slender, articulate below middle, articulation swollen and slightly inflexedIn fruit. Outer tepals oblong, ca. 1.5 mm; inner tepals enlarged in fruit; valves broadly cordate, 6-7 mm, all or 1 or 2 valves with narrowly ovate tubercles (in *R. patientia* s.str. normally 1 valve has a large tubercle, and two other valves have smaller tubercles), net veined, base deeply cordate, margin entire or indistinctly erose, apex obtuse. Achenes brown, shiny, ovoid, trigonous, 2.5-3 mm, apex acuminate.

Rumex thianschanicus[1] *:* Herbs perennial. Stems erect, 70.130 cm tall, robust, branched, glabrous, grooved. Basal leaves shortly petiolate,broadly ovate, 14.28 × 7.17 cm, thin, both surfaces glabrous, abaxially with prominent veins, base cordate, margin slightly undulate, apex subacute; cauline leaves shortly petiolate, small; ocrea fugacious, membranous. Inflorescence paniculate, lax. Flowers bisexual. Pedicel filiform, 8.16 mm, slender, dilated upward, articulate near base. Inner tepals enlarged in fruit; valves broadly cordate, 5.7 × 6.8 mm, only 1 valve with a tubercle, net veined, base cordate, margin nearly entire, apex acuminate; tubercle elliptic, 2.3 mm. Achenes brownish, ovoid,trigonous, 2.3 mm, apex acuminate.

Rumex crispus[1]*:* Herbs perennial. Roots large. Stems erect, 50-120(-150) cm tall, simple or branched above, glabrous, grooved. Basal leaves shortly petiolate, lanceolate or narrowly lanceolate, 10-25 × 2-5 cm, glabrous or indistinctly papillose along veins below, base usually cuneate to truncate, margin strongly crisped and undulate, apex acute; cauline leaves shortly petiolate, narrowly lanceolate, small; ocrea fugacious, membranous. Inflorescenceterminal, paniculate, narrow; branches erect or ascending. Flowers bisexual. Pedicel slender, articulate in proximal third, articulation distinctly swollen. Inner tepals enlarged in fruit; valves broadly ovate, 3.5-6 × 3-5 mm, all with tubercles, rarely only 1 valve bearing a tubercle, conspicuously net veined, base nearly truncate, margin entire, rarely weakly erose, apex obtuse to subacute; tubercle ovate, 1.5-2 mm. Achenesdark brown, shiny, ovoid, trigonous, ca. 2 mm, apex acute.

Rumex confertus[1]*:*Herbs perennial. Stems erect, 40-50 cm tall, branched above, grooved, papillose-pubescent. Basal leaves with petiole longer than leaf blade; leaf blade deeply cordate-triangular, 8-10 × 15-20 cm, slightly longer than wide, abaxially papillate, adaxially glabrous, margin undulate, basal lobes and apex rounded. Inflorescence paniculate, 5-6 × 18-20 cm; rachis flexuous; branches arcuate at base. Flowers bisexual. Pedicel slender, articulate below middle. Inner tepals enlarged in fruit; valves broadly cordate, acutely reniform, 5-6 × 7-8 mm, one valve with a small tubercle, conspicuously net veined, margin with indistinct teeth near base.

Rumex japonicus[1]: Herbs perennial. Stems erect, 50-100 cm tall, branched above, grooved, glabrous. Basal leaves: petiole 6-15 cm; leaf blade oblong or lanceolate-oblong, 8-25 × 3-8 cm, abaxially minutely papillate along veins, adaxially glabrous, base rounded, cordate, or broadly cuneate, margin slightly undulate, apex acute or obtuse; cauline leaves shortly petiolate, narrowly oblong, small; ocrea fugacious, white, membranous. Inflorescence paniculate. Flowers bisexual. Pedicel slender, articulate below middle, articulation distinct.

Inner tepals enlarged in fruit; valves broadly cordate, 4-5 × 5-6 mm, all valves with narrowly ovate tubercles, conspicuously net veined, base cordate, margin irregularly denticulate, apex acute; denticles 0.3-0.5 mm. Achenes dark brown, shiny, broadly ovoid, sharply trigonous,ca. 2.5 mm, base narrow, apex acute.

Rumex stenophyllus[1]*:* Herbs perennial. Roots vertical, large, to 1 cm in diameter. Stems erect, 40-80(-120) cm tall, usually branched above, glabrous, grooved. Basal leaves shortly petiolate, lanceolate or narrowly lanceolate, 10-18 × 1.5-4 cm, glabrous or indistinctly papillose along veins below, base cuneate, margin crisped, occasionally nearly flat and entire, apex acute; cauline leaves shortly petiolate or nearly sessile, narrowly lanceolate, small; ocrea fugacious, membranous. Inflorescence paniculate, narrow. Flowers bisexual, dense. Pedicel slender, articulate below middle (in proximal third). Inner tepals enlarged in fruit; valves triangular, 3-4(-5) mm × ca. 3.5 mm, all valves with narrowly ovate tubercles, base truncate to indistinctly cordate, margin denticulate, apex acute; denticles 0.5-1.5 mm, 4-10 at each side. Achenes brown, shiny, ellipsoid, 2.5-3 mm, sharply trigonous, base narrow, apex acute.

Rumex obtusifolius[1]*:*Herbs perennial. Roots vertical, large, to 1.5 cm in diameter. Stems erect, 60-120(-150) cm tall, grooved, branched above middle or in upper 2/3, glabrous. Basal leaves: petiole 6-12 cm, minutely papillate; leaf blade broadly ovate to oblong-ovate or narrowly ovate, 15-30 × 6-15 cm, base cordate, abaxially sparsely minutely papillate, adaxially glabrous; cauline leaves shortly petiolate, narrowly ovate, small; ocrea fugacious, membranous. Inflorescence broadly paniculate, large; branches ascending. Flowers bisexual, dense. Pedicel filiform, slender, articulate below middle (in proximal third, rarely near middle). Inner tepals enlarged in fruit; valves narrowly triangular-ovate,4-6 × 2-3 mm, usually 1 valve with tubercles, sometimes 3 valves with tubercles, but then 1 tubercle distinctly larger than other 2, base truncate, each margin with 2 or 5 teeth, apex obtuse to subacute; teeth 0.8-1.5 mm, apex straight. Achenesdark brown, shiny, ovoid, sharply trigonous, ca. 2.5 mm.

Rumex chalepensis[1]*:* Herbs perennial. Roots black-brown, large, to 2.5 cm in diameter. Stems erect, 30-60 cm, grooved, branched. Basal leaves: petiole 3-4 cm; leaf blade oblong, 5-20 × 3-8 cm, both surfaces glabrous, midvein prominent abaxially, base rounded or subcordate, margin slightly undulate, apex obtuse or acute; cauline leaves shortly petiolate, small; ocrea fugacious, membranous. Inflorescence paniculate, large. Flowers bisexual. Pedicel articulates below middle. Outer tepals elliptic; inner tepals enlarged in fruit; valves triangular-cordate, 5-6 mm, all valves with tubercles, conspicuously net veined, base subcordate, margin denticulate, apex acute; denticles 1-1.5 mm;tubercles oblong, ca. 2 mm. Achenes brown, shiny, ellipsoid,
Sharply trigonous, 2.5-3 mm, base narrow, apex acute.

Rumex nepalensis[1]*:* Herbs perennial. Roots large. Stems erect, 50-100 cm tall, branched above, glabrous, grooved. Basal leaves: petiole 4-10 cm; leaf blade broadly ovate, 10-15 × 4-8 cm, both surfaces glabrous or abaxially minutely papillate along veins, base cordate, margin entire, apex acute; cauline leaves shortly petiolate, ovate-lanceolate; ocrea fugacious, membranous. Inflorescence paniculate. Flowers bisexual. Pedicel articulates below middle. Outer tepals elliptic, ca. 1.5 mm; inner tepals enlarged in fruit; valves broadly ovate, 5-6 mm, valves all or 1 or 2 withtubercles, base truncate, each margin with 7 or 8 teeth, apex acute; teeth 1.5-3 mm, apex hooked or straight. Achenesbrown, shiny, ovoid, sharply trigonous, ca. 3 mm, base truncate,apex acute

Rumex dentatus[1]*:* Herbs annual, rarely biennial. Stems erect, 30-70 cm tall, branched from base, grooved; branches ascending to nearly divaricate, glabrous. Lower leaves: petiole 3-5 cm; leaf blade oblong to narrowly elliptic, 4-12 × 1.5-3 cm, both surfaces glabrous, or papillose along veins below, base rounded, truncate, or subcordate, margin slightly undulate, apex obtuse or acute; cauline leaves smaller; ocrea fugacious, membranous. Inflorescence racemose, several racemes aggregated and panicle-like. Flowers bisexual. Pedicel articulates below middle (in proximal third). Outer tepals elliptic, ca. 2 mm; inner tepals enlarged in fruit; valves triangular-ovate, 4-5 × 2.5-3 mm, allvalves with tubercles 1.5-2 mm (in some infraspecific taxa of *R. dentatus* only 1 or 2 valves with tubercles), conspicuously net veined, base rounded, each margin with 2-4 teeth, apex acute to subacute; teeth 1.5-2 mm. Achenes yellow-brown,shiny, ovoid, sharply trigonous, 2-2.5 mm, base narrow, apexacute.

Rumex trisetifer[1]: Herbs annual. Roots large. Stems erect, 30-80 cm tall, grooved, glabrous; branches spreading. Lower leaves: petiole 3-5 cm; leaf blade oblong or lanceolate-oblong, 8-20 × 2-5 cm, both surfaces glabrous, base cuneate, margin undulate, apex acute; cauline leaves shortly petiolate, narrowly lanceolate, smaller than basal ones; ocrea fugacious, membranous. Inflorescence terminal or axillary, racemose, several racemes aggregated and large panicle-like. Flowers bisexual. Pedicel slender, articulate near base. Outer tepals lanceolate, small; inner tepals enlarged in fruit; valves narrowly trigonous-ovate, 3-4 × 1.5-2 mm, all valves with tubercles, base truncate, marginwith 1 pair of narrow teeth, apex narrowly acute; teeth 3-4 mm, straight. Achenes yellow-brown, shiny, ellipsoid, sharply trigonous, 1.5-2 mm, base narrow, apex acute.

Rumex maritimus[1]*:*Herbs annual, rarely biennial, especially in South regions. Stems erect, 15-60 cm tall, branched below middle, grooved, glabrous or weakly shortly papillose. Lower leaves: petiole 1-2.5 cm; leaf blade lanceolate or lanceolate-oblong, 4-15-(-20) × 1-3-(-4) cm, both surfaces glabrous or shortly papillose below, base narrowly cuneate, margin entire and smooth, or occasionally slightly undulate, apex acute, cauline leaves shortly petiolateor nearly sessile, smaller than basal ones; ocrea fugacious, membranous. Inflorescence paniculate. Flowers bisexual. Pedicel filiform, articulate at base or slightly above base, articulation indistinctly swollen. Outer tepals elliptic, ca. 2 mm; inner tepals enlarged in fruit; valves narrowly triangular-ovate, 2.5-3.5 × 0.8-1.5 mm wide, all valves with tubercles, base truncate, each margin with 2 or 3(or 4) teeth, apex acute; teeth 2.5-3 mm, narrow; tubercles oblong, ca. 1.5 mm.Achenes yellowbrown,shiny, ellipsoid, sharply trigonous, 1.5-2 mm.

Rumex similans[1]*:* Herbs annual. Stems erect, purplish red, 15-30 cm tall, branched from base, finely grooved. Lower leaves: petiole 1-3 cm; leaf blade oblong or lanceolate-oblong, 3-7 × 0.8-2 cm, both surfaces glabrous, with conspicuous midvein, base rounded or broadly cuneate, margin slightly crisped, apex acute, cauline leaves shortly petiolate or nearly sessile, small, upper ones linear-lanceolate; ocrea fugacious, membranous. Inflorescence terminal, racemose, leafy. Flowers bisexual. Pedicel articulates at base.Outer tepals lanceolate, ca. 0.5 mm; inner tepals enlarged in fruit; valves triangular-ovate, 2-2.5 × 1-1.5 mm, all valves with tubercles, base rounded, each margin with 3 or 4 pairs of narrow teeth, apex narrowly acute; teeth 1-1.5 mm. Achenes shiny, ovoid, 1-1.5 mm, sharply trigonous, apex acute.

Rumex marschallianus[1]: Herbs annual. Stems erect, 10-30(-50) cm tall, branched from base, glabrous, finely grooved.

Lower leaves: petiole 1-1.5 cm, slender; leaf blade lanceolate or elliptic-lanceolate, 1.5-5 × 0.7-1.5 cm, both surfaces glabrous, midvein conspicuous, base cuneate or rounded, margin slightly crisped, apex acute;cauline leaves small, with short petiole 3-5 mm. Inflorescence racemose, several racemes aggregated and panicle-like, leafy. Flowers bisexual. Pedicel slender, articulate at base. Outer tepals elliptic; inner tepals enlarged in fruit; valves ovate-triangular, 2.5-3 mm, only 1 valve with tubercle, base rounded, each margin with 2 or 3 narrow teeth (ca. 1.5 or)4-5 mm, apex narrowly acute; others without tubercles, with shorter teeth, orall valves with subequal teeth.Achenes brown, shiny, ovoid,sharply trigonous, ca. 1 mm, base truncate, apex acute.

3. **Chemical Constitutents of Different Species of*Rumex:***

Six *C*-glucosyl anthrones are identified as Rumejaposide E (10*R*-*C*-β-d-glucosyl-10-hydroxyemodin-9-anthrone) and Rumejaposide F (10*S*-*C*-β-d-glucosyl-10-hydroxyemodin-9-anthrone),rumejaposide G (10*R*-*C*-β-d-glucosylemodin-9-anthrone) and Rumejaposide H, (10*S*-*C*-β-d-glucosylemodin-9-anthrone),Cassialoin,(10*S*-*C*-β-d-glucosyl-10hydroxychrysophanol-anthrone)andRumejaposideI**(**10*R*-*C*-β-d-glucosyl-10 hydroxychrysophanol-9-anthrone) isolated from the roots of *Rumex dentatus* by column chromatography[5]. Ten compounds were obtained and identified as Helonioside A, Gallicacid, Isovanillic acid, p-hydroxycinnamic acid, Succinic acid, n-butyl-beta-D-fructopyranoside, Quercetin, Hexadecanoic acid 2, 3-dihydroxy propyl ester, beta-sitosterol and Daucosterol from the roots of *Rumex dentatus*[6].

Two stilbene-O-glycosyl derivatives were Piceid(5,4'-dihydroxystilbene-3-O-beta-d-glucopyranoside) and Rumexoid (5,4'-dihydroxystilbene-3-O-alpha-arabinopyranoside) in addition to Resveratrol (3,5,4'-trihydroxystilbene) isolated from roots of rumex bucephalophorus[7].Two stilbene-O-methyl derivatives were 5,4'-dihydroxy-3-methoxystilbene and 3,5-dihydroxy-4'-methoxystilbene in addition to resveratrol (3,5,4'-trihydroxystilbene) isolated from the same plant[8].

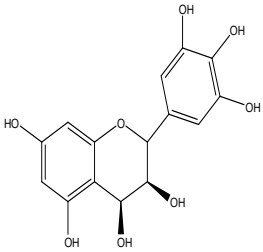
1,5-Dihydroxyanthraquinones and an Anthrone from Roots of *Rumex Crispus*[9].The separation of 1,5-dihydroxy-3-methyl anthraquinone;1,3,5-trihydroxy-6 hydroxymethyl anthraquinone;1,5-dihydroxy-3-methoxy-7-methyl anthraquinone by micellar electrochromatographic method from the root of *Rumex crispus* [10].

Two known compounds as 1-O-beta-D-glucopyranosyl chrysophanol and 1-O-beta-D-glucopyranosyl emodin were isolated from the methanol extract of root of Rumex gmelini[11].From the same plant ten compounds were identified as Nepodin, Emodin, Citreorosein, Chrysophanol 8-O-β-(6'-acetyl) glucopyranoside, Chrysophanol 8-O-β-D-glucopyranoside,

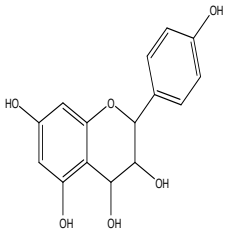
Resveratrol,9,9'-dianthranone-2,2'-dimethyl-5,5'-bis (β-D-glucopyranose)-9,9',10,10'-tetrahydro-4,4'-dihydroxy-10,10'-dioxo (trivial name:rumoside A) ,Emodin-8-O-β-D-glucopyranoside,Resveratrol-3-O-β-D-glucoside and Rutin[12].

A new chromone glucoside 2,5-dimethyl-7-hydroxychromone-7-O-β-glucopyranoside isolated from the 75% EtOH extract of the roots of Rumex gmelini Turcz., together with five known compounds, nepodin-8-O-β-D-glucopyranoside, 10-hydroxyaloin A , 10-hydroxyaloin B , 5-methoxyl-1(3H)-benzofuranone-7-O-β-D-glucopyranoside,

phenylethyl-O-α-L-arabinopyranosy- (1→6)- O-β-D-glucopyranoside[13] . The identification of leucodelphinidin and leucopelargonidin from *Rumex hymenosepalus from* the antitumour fractionof ethanolic extract.[14]

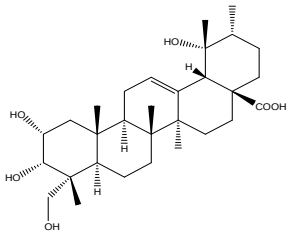


LEUCODELPHINIDIN

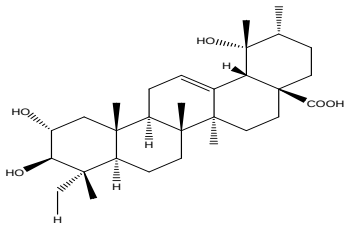


LEUCOPELARGONODIN

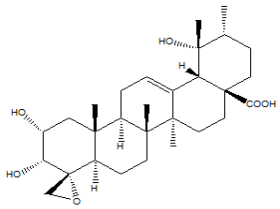
Four ursane-type triterpenoids, 2α,3α,19α-trihydroxy-24-norurs-4(23),12-dien-28-oic acid , 4(R),23-epoxy-2α,3α,19α-trihydroxy-24-norurs-12-en-28-oic acid , myrianthic acid and tormentic acid , were isolated from an EtOAc solubleextract of the stems of *Rumex japonicas*[15].



Myrianthic acid

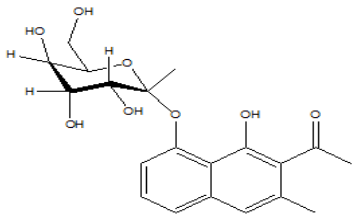


Tormentic acid

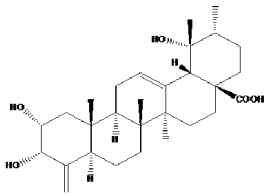


4(R), 23-epoxy- 2α,3α,19α-trihydroxy-24-norurs-12-en-28-oic acid

Musizin-8-O-*β*-D-glucoside isolated from the same plant[16].

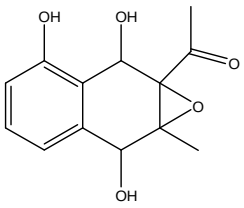


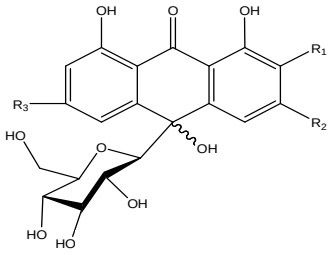
Musizin-8-O-*β*-D-glucoside



2α,3α,19α-trihydroxy-24-norurs-4 (23),12-Dien-28- oic acid.

Five oxanthrone *C*-glycosides, namely rumejaposide A–E, and an epoxynaphthoquinol were isolated from roots of *Rumex japonicus* [17]

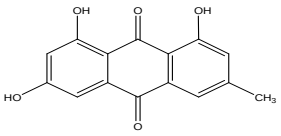




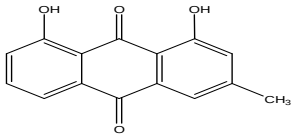
Epoxynaphthoquinol

Rumejaposide	R_1	R_2	R_3
A (10R)	COOH	CH_3	H
B (10S)	COOH	CH_3	H
C (10R)	COOH	CH_3	OH
D (10R)	H	CH_2OH	OH
E (10R)	H	CH_3	OH

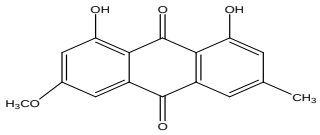
Three anthraquinones—emodin, chrysophanol, and physcion—were successfully Purified from the dichloromethane extract of the Chinese medicinal herb *Rumex japonicus* By high-speed counter-current chromatography[18]



Emodin

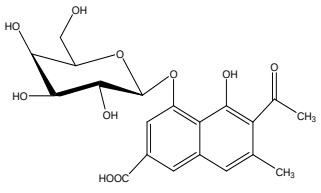


Chrysophanol

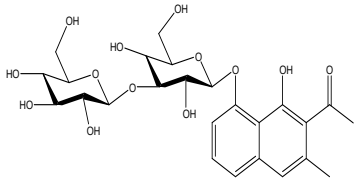


Physcion

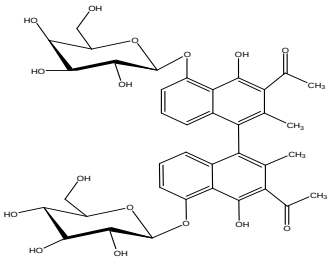
The structures of the new compounds were established, respectively as rumexoside (2-acetyl-3-methyl-6-carboxy-1,8 dihydroxynaphthalene-8-O-β-D-glucopyranoside), labadoside (4,4''-binaphthalene-8,8''-O,O-di-β-D-glucopyranoside) and orientaloside(2-acetyl-3-methyl-1,8-dihydroxynaphthalene-8-O-β-D-glucopyranosyl (1→3) β-D-glucopyranoside) on the basis of spectral analysis were isolated from the roots of *Rumex patientia* L[19].



Rumexoside

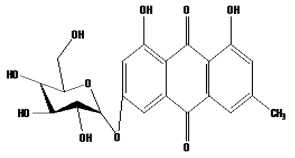


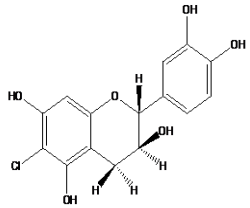
Orientaloside



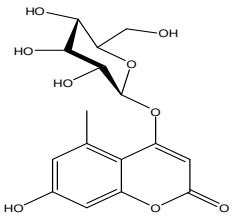
Labadoside

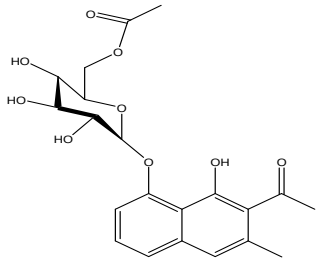
Two new naphthalene derivatives, named patientosides A andB were isolated from the roots of *Rumex patientia*[20]. An anthraquinone glycoside, emodin-6-*O*-β-D -glucopyranoside and a simple halogenated flavan-3-ol, 6-chlorocatechin have been isolated from the same plant[21].





Anhydrolutein I (= (all-E,3R,6'R)-3',4'-didehydro-beta,gamma-caroten-3-ol; 2) and anhydrolutein II (= (all-E, 3R,6'S)-2',3'-didehydro-beta,epsilon-caroten-3-ol; 3) have been isolated and characterized from the extract of steam-cooked sorrel.(*Rumex rugosus*)[22].





Hastatusides A and B: Two New Phenolic Glucosides from *Rumex hastatus*[23]

Table-1:-Traditional and Medicinal Uses: [24-28]

Plant	Part used	Uses
Rumex vesicarius	Leaf	Stomachic,Diuretic,Astringent,Aperient
Rumex acetosella	Leaf,Fresh plant juice	Cancer,Antiscorbutic,Refrigerant,Diuretic
Rumex crispus	Root and Seed	Dentrifrice,Chronic dysentery and nausea,Hepatic disorders
Rumex dentatus	leaf Stem and Root	Antibacterial,Cytotoxicity,Antitumour,Antiscorbutic,Cutaneous disorders and used as dye
Rumex maritimus	Leaf and root	Cathartic,PurgativeAntipruritic,CNSdepressant, Antidiarrhoeal and applied to burns
Rumex nepalensis	Infusion of leaves	Syphilitic ulcersand purgative
Rumex scutatus	Leaf	Astringent,Refringent and Antiscorbutic
Rumex acetosa	Leaf and infusion of roots	Refringent,Diuretic and skin troubles
Rumex bucephalophorus	Roots	Antioxidant
Rumex confertus	Whole plant or Root	Detoxification,Defaecation and Insecticide
Rumex ecklonianus	Leaves	Purgative,Treatment of chlorosis and anemia
Rumex gmelini	Leaves	Anti-asthmatic,Antitussive,Antitumour and Antioxidant
Rumex japonicus	Aerial parts,Root	Antioxidant and Antimicrobial,Apoptosis
Rumex patentia	Seeds,Root	Antihyperglycemic,Antihyperlipidemic, Antiinflammatory
Rumex abyssinicus	Root	Antiinflammatory,Analgesic and Antihelminthic

4. **Conclusion:**

There are many species of Rumex which grow in different parts of the world of which only a few of them were reported based on the chemical constituents and activities . The reviews indicated that Rumex Sps. consist mostly of flavonoids and anthraquinones which are responsible for the different pharmacological activities of the members of this genus.

Table: 2:- Different Species of Rumex through Worldwide

Rumex acetosa	*Rumex balcanicus*	*Rumex densiflorus*	*Rumex lorentzianus*
Rumex acetosella	*Rumex brownie*	*Rumex dentatus*	*Rumex x lousleyi*
Rumex x acutus	*Rumex brownie*	*Rumex diclinis*	*Rumex ludovicianus*
Rumex albescens	*Rumexbucephalophorus*	*Rumex digynus*	*Rumex lugdunensis*
Rumex x alexidis	*Rumex chrysocarpus*	*Rumex dimidiatus*	*Rumex lunaria*
Rumex alpestris	*Rumex confertus Willd.*	*Rumex dimorphophyllus*	*Rumex luxurians*
Rumex alpinus	*Rumex x confuses*	*Rumex x dissimilis*	*Rumex x lycheanus*
Rumex altissimus	*Rumex conglormeratus*	*Rumex x dobrogensis*	*Rumex maderensis*
Rumex angiocarpus	*Rumex costaricensis*	*Rumex x dolosus*	*Rumex magellanicus*
Rumex aquaticus	*Rumex crispus*	*Rumex dregeanus*	*Rumex maritimus*
Rumex aquitanicus	*Rumex cristatus*	*Rumex drobovii*	*Rumex polycarpus*
Rumex azoricus	*Rumex crystallinus*	*Rumex drummondii*	*Rumex polygamous*
Rumex × dufftii	*Rumex flexicaulis*	*Rumex gussonii*	*Rumex polyklonos*
Rumex dumosiformis	*Rumex flexuosiformis*	*Rumex x gusuleacii*	*Rumex* x *promiscuous*
Rumex dumosus	*Rumex foliosus*	*Rumexhadmocarpus*	*Rumex* x *propinquus*
Rumexdumulosus	*Rumexfontanopaludosus*	*Rumex halophilus*	*Rumex protractus*
Rumexdurispissimus	*Rumex foveolatus*	*Rumex hararensis*	*Rumex pseudonatronatus*
Rumex ecklonianus	*Rumex x franktonis*	*Rumex hasslerianus*	*Rumex* x *pseudopulcher*
Rumexecuadoriensis	*Rumex fraternus*	*Rumex hastatulus*	*Rumex pseudoscutatus*
Rumex elbrusensis	*Rumexfringillimontanus*	*Rumex hastatus*	*Rumex pseudoxyria*
Rumex ellenbeckii	*Rumex frutescens*	*Rumex hayekii*	*Rumex pulcher*
Rumex engelmanni	*Rumex fueginus*	*Rumexhazslinszkyanus*	*Rumex quarrei*
Rumexephedroides	*Rumex gamsii*	*Rumex x heimerlii*	*Rumex raulini*
Rumex erosus	*Rumex gangotrianus*	*Rumex hellenicus*	*Rumex rechingerianus*
Rumex erubescens	*Rumex gieshueblensis*	*Rumex henrardi*	*Rumex rectinervius*
Rumexerythrocarpus	*Rumex giganteus*	*Rumex hesperius*	*Rumex recurvatus*
Rumex esquirolii	*Rumex ginii*	*Rumex heteranthos*	*Rumex* x *rhaeticus*
Rumex euxinus	*Rumex gmelini*	*Rumex heterophylus*	*Rumex rhodesius*
Rumexevenkiensis	*Rumex gombae*	*Rumex hexagynus*	*Rumex x romanicus*
Rumexexspectatus	*Rumex gracilescens*	*Rumex hippiatricus*	*Rumex romassa*
Rumex fallacinus	*Rumex gracilipes*	*Rumex hirsutus*	*Rumex* x *rosemurphyae*
Rumexfascicularis	*Rumex graminifolius*	*Rumex horizontalis*	*Rumex roseus*
Rumex fascilobus	*Rumex granulosus*	*Rumex hoschedei*	*Rumex rossicus*
Rumex fimbriatus	*Rumex x griffithii*	*Rumex hostilis*	*Rumex rothschildianus*
Rumex finitimus	*Rumex x grintzescui*	*Rumex hultenii*	*Rumex rugosus*
Rumexhungaricus	*Rumex x hybridus*	*Rumexhydrolapathum*	*Rumex rupestris*
Rumex hymenosepalus	*Rumex interruptus*	*Rumex kaschgaricus*	*Rumex ruwenzoriensis*
Rumex x impurus	*Rumex x inundates*	*Rumex x kaschmirianus*	*Rumex sagittatus*
Rumex inconspicuous	*Rumex iseriensis*	*Rumex kerneri*	*Rumex x sagorski*
Rumex integer	*Rumex jacutensis*	*Rumex khekii*	*Rumex salicetorum*
Rumex integrifolia	*Rumex japonicas*	*Rumex x khorasanicus*	*Rumex salicifolius*
Rumex x intercedens	*Rumex x johannis-moorei*	*Rumex x knafii*	*Rumex salinus*
Rumex intermedius	*Rumex kamtshadalus*	*Rumex komarovii*	*Rumex samuelssoni*
Rumex krausei	*Rumex marschallianus*	*Rumex obovatus*	*Rumex sanguineus*
Rumex lachanus	*Rumex maximus*	*Rumex obtusifolius*	*Rumex sanninensis*
Rumex lacustris	*Rumex megalophyllus*	*Rumex occidentalis*	*Rumex suzukianus*
Rumex lanceolatus	*Rumex meyeri*	*Rumex occultans*	*Rumex vesceritensis*
Rumex langloisii	*Rumex × mezei*	*Rumex ochotensis*	*Rumex vesicarius*
Rumex lanuginosus	*Rumex microcarpus*	*Rumex orbiculatus*	*Rumex violascens*
Rumex lapponicus	*Rumex microdon*	*Rumex orientalis*	*Rumex wachteri*
Rumex lanuginosus	*Rumex x mirabilis*	*Rumex orthoneurus*	*Rumex x weberi*
Rumex latifolius	*Rumex mixtus*	*Rumex x oryzetorum*	*Rumex longifolius*
Rumex lativalvis	*Rumex moedlingensis*	*Rumex osswaldii*	*Rumex longisetus*
Rumex leptocaulis	*Rumex x monistrolensis*	*Rumex oxysepalus*	*Rumex x nankingensis*
Rumex leptophyllus	*Rumex montanus*	*Rumex x pakistanicus*	*Rumex natalensis*
Rumex limoniastrum	*Rumex monticola*	*Rumex pallidus*	*Rumex neglectus*
Rumex linearis	*Rumex muelleri*	*Rumex palustris*	*Rumex nematopodus*
Rumex x lingulatus	*Rumex x munshii*	*Rumex x palustroides*	*Rumex nemorosus*
Rumex litoralis	*Rumex muretii*	*Rumex pamiricus*	*Rumex nepalensis*
Rumex lonaczewskii	*Rumex muricatus*	*Rumex x pannonicus*	*Rumex nervosus*

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